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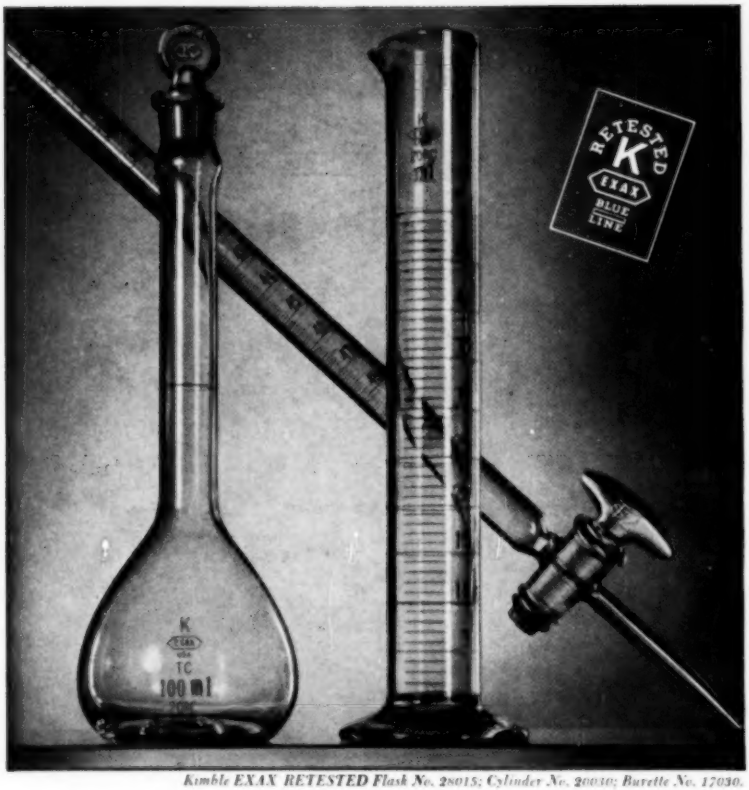

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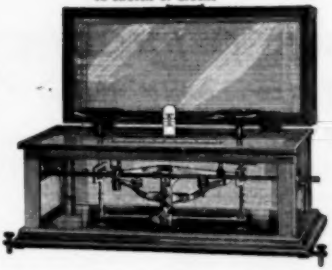

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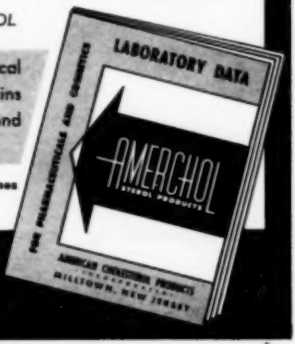

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VOLUME XXXVIII | *JULY, 1949* | NUMBER 7 | CONSECUTIVE NO. 14

Synthesis of Certain Derivatives of Thiols*,†

By JOHN W. BOENIGK,‡ JOHN E. CHRISTIAN, and GLENN L. JENKINS

Seven aromatic thiols have been used to prepare two series of sulfides. Thiols used were *o*-thiophenol, *o*-thiocresol, *m*-thiocresol, *p*-thiocresol, *o*-chlorobenzyl thiol, 2,4-dichlorobenzyl thiol, and 3,4-dichlorobenzyl thiol. A series was prepared of 3-diethylaminopropyl derivatives and 3-diethylamino-2-propanol derivatives. The compounds are analogous to Benadryl and Pyribenzamine type compounds with sulfur replacing the oxygen and nitrogen, respectively. The general structure of the molecule differs from these antihistamine drugs in that only one aryl group is present instead of the diaryl groups of Benadryl and Pyribenzamine. The aryl structure was varied to show the influence of substituent groups, in order that some of the thiols used would contain chlorine on the ring; others would contain methyl groups.

THE PURPOSE of the research project was to synthesize sulfur compounds analogous to Benadryl and Pyribenzamine type compounds with sulfur replacing the oxygen and nitrogen linkage, respectively. The general structure of the molecule differs from these antihistamine drugs in that only one aryl group is present instead of the diaryl groups of Benadryl and Pyribenzamine. The aryl structure was varied to show the influence of substituent groups, in order that some of the thiols used would contain chlorine on the ring; others would contain methyl groups.

It was planned to prepare two general groups of thiol derivatives, one in which the substituent was 3-diethylaminopropyl, and the other in which the substituent was 3-diethylamino-2-propanol. The diethylaminopropyl substituent has been shown to have neurotropic activity (1) and the sulfur linkage to have musculotropic activity (2), the combination of the side chain and the sulfur may have desirable antispasmodic or antihistamine activity. The purpose of the second side chain was to test the influence of such a secondary alcohol.

METHOD OF PREPARATION

The thiols used in the research project were either commercially available or were synthesized by either reduction of the corresponding disulfide or reaction of an appropriate benzyl chloride with thiourea (3). (See Tables I and II.)

* Received April 4, 1949, from the Research Laboratories, Purdue University, School of Pharmacy, Lafayette, Ind.
Presented to the Scientific Section, A. PH. A., Jacksonville meeting, April, 1949.
† The work reported in this paper was made possible through a grant from the American Foundation for Pharmaceutical Education.
‡ Present address: The University of Texas, School of Pharmacy, Austin, Tex.

The 3-diethylaminopropyl derivatives were prepared according to the following sequence of reactions:

1. $Ar—SH + KOH \rightarrow Ar—S—K + H_2O$
2. $Ar—S—K + Cl—CH_2—CH_2—CH_2—N(C_2H_5)_2 \cdot HCl + KOH \rightarrow Ar—S—CH_2—CH_2—CH_2—N(C_2H_5)_2 + 2KCl + H_2O$
3. $Ar—S—CH_2—CH_2—CH_2—N(C_2H_5)_2 + HCl \rightarrow Ar—S—CH_2—CH_2—CH_2—N(C_2H_5)H_2 \cdot Cl$

The 3-diethylamino-2-propanol derivatives were prepared as follows:

1. $Ar—SH + NaOH \rightarrow Ar—SNa + H_2O$
2. $Ar—SNa + \underset{\diagdown\,O\,\diagup}{H_2\ H\ H_2\ \ \ \

TABLE III.—DIETHYLAMINOPROPYL SULFIDE HYDROCHLORIDES

Thiol Used	B. P., ° C./Mm. Amine	Yield Amine, Gm.[a]	M. P., ° C. Amine HCl[b]	Nitrogen[b] Calcd.	Nitrogen[b] Found	Cl[c] as HCl Calcd.	Cl[c] as HCl Found
o-Chlorobenzyl thiol	190–193/12	20 (80%)	83–84	4.54	4.46	11.49	11.45
2,4-Dichlorobenzyl thiol	165–168/4	22.5 (73%)	116–117	4.08	4.02	10.31	10.29
3,4-Dichlorobenzyl thiol	169–172/3	24.9 (81%)	75–76	4.08	4.03	10.31	10.24
Thiophenol	141–143/6	18.1 (81%)	61–63	6.26	5.90[d]	13.67	13.15
o-Thiocresol	150–153/5	16.9 (71%)	126–127	5.11	4.93	12.95	12.98
m-Thiocresol	168–173/13	20.8 (88%)	65–66	5.90	5.41[d]	12.95	12.52
p-Thiocresol	155–158/7	19.4 (81%)	97–98	5.11	5.08	12.95	12.90

[a] Recrystallizing solvent: ethyl acetate.
[b] Nitrogen determined by a semimicro Kjeldahl method (7).
[c] Chlorine determined by the method described by Blicke and Zienty (6).
[d] Amine hydrochlorides were very hygroscopic, N analysis made on free base.

the oil separated, and the aqueous layer extracted with ether. The combined ether extracts and oil were dried with anhydrous K_2CO_3. After evaporation of the ether, the residual oil was distilled under reduced pressure. The yield obtained was 59 Gm. (49% theoretical), the boiling point was 68–70° at 20 mm.

Method B.—A solution of γ-diethylaminopropanol 78.5 Gm. (0.6 mole) in 130 cc. of chloroform was added slowly with cooling to a solution of thionyl chloride 145 Gm. (1.21 mole) in 600 cc. of chloroform. The mixture was refluxed for three hours, after which the solvent and excess thionyl chloride were removed by distillation. The residue was treated with 150 cc. of a 40% solution of NaOH and extracted with 1000 cc. of ether. The ether solution was dried over anhydrous Na_2SO_4 and the ether evaporated. The residue distilled as a colorless oil 62–64° at 19 mm., yield 79.3 Gm. (88% theoretical). Gilman and Shirley (9) reported a yield of 73% theoretical, boiling point 73–75° at 20 mm.

1-Diethylamino-2,3-epoxypropane was also prepared by two methods (10, 11).

Method A.—A mixture of epichlorohydrin (46.3 Gm.), diethylamine (30.0 Gm.) and water (1.0 Gm.) was well mixed while maintaining the temperature at 28–30° for four hours. The resultant oil was shaken with 50 cc. 20% K_2CO_3, then with 60 cc. of a 35% NaOH solution for one hour. The oil was then shaken with 50 cc. of a 50% KOH solution. The resultant oil after separation from the KOH solution was distilled under reduced pressure. The yield obtained was 35.8 Gm. (55.4% theoretical). The boiling point of the product was 40–50° at 8 mm.

Method B.—A mixture of epichlorohydrin (111.2 Gm.), diethylamine (86.4 Gm.), and 3.6 Gm. water was stirred vigorously for six hours maintaining the temperature of the reaction mixture at 28–30°. The reaction mixture was then cooled to 20° and a solution of NaOH (50 Gm. in 91 cc. H_2O) was added slowly, meanwhile maintaining vigorous stirring. The reaction mixture was stirred for an additional forty minutes and poured into 200 cc. water. The top layer was separated and the aqueous layer extracted with ether. The ether extracts plus the oil were dried over KOH pellets. The ether layer was separated from the KOH and the ether removed by evaporation. The residual oil distilled at 53–56° at 17 mm., the yield being 75 Gm. (50% of theoretical). Gilman reported a 62% yield and the boiling point as 62–65° at 20 mm.

The compounds listed in Table IV were prepared as follows: The sodium hydroxide 8.8 Gm. (0.2 mole plus 10% excess) was dissolved in 50 cc. of water and the thiol (0.2 mole) added to the NaOH solution. The 1-diethylamino-2,3-epoxypropane 25.8 Gm. (0.2 mole) was dissolved in 50 cc. of water, and added slowly with vigorous stirring to the thiol-NaOH solution. The mixture was stirred for an additional four hours, and the resultant oily product after being washed with water, was dissolved in 50 cc. of benzene. The benzene solution was dried with anhydrous sodium sulfate and the benzene removed by evaporation under reduced pressure. The residual oil was distilled under reduced pressure. All attempts to convert these amines to crystalline hydrochlorides failed.

SUMMARY

1. Seven diethylaminopropyl and seven diethylaminohydroxypropyl derivatives of thiols have been prepared and characterized.

TABLE IV.—1-DIETHYLAMINO-2-HYDROXY-PROPYLSULFIDES

Thiol Used	B. P., ° C./Mm.	Yield Amine, Gm.	Nitrogen[a] Calcd.	Nitrogen[a] Found	Refractive Index (/° C.) Amine
o-Chlorobenzyl thiol	190–195/4	17 (60%)[b]	4.85	4.56	1.5435/30
2,4-Dichlorobenzyl thiol	210–215/4	42.8 (66%)	4.34	4.23	1.5522/27
3,4-Dichlorobenzyl thiol	210–215/4	44.3 (69%)	4.34	4.14	1.5515/30
Thiophenol	168–171/4	41.3 (86%)	5.85	5.79	1.5412/25
o-Thiocresol	154–157/1	35.6 (65%)	5.50	5.32	1.5408/30
m-Thiocresol	168–172/3	32.0 (60%)	5.50	5.37	1.5376/30
p-Thiocresol	168–172/3	35.0 (64%)	5.50	5.28	1.5371/30

[a] Nitrogen determined by semimicro Kjeldahl method (7).
[b] Made using 0.1 molar quantities, others made using 0.2 molar quantities.

2. Sufficient quantities of these compounds have been prepared for pharmacological study.

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Assay of Heparin. III. Measurement of the End Point by Physical Means. Influence of Surface on Clotting*,†

By R. H. K. FOSTER

In the assay of heparin using beef or sheep plasma the degree of clotting has been estimated by visual inspection. This estimate, visual density, has been correlated with light transmission as measured photoelectrically and with the weights of dried clots. Good agreement was obtained. At times the surface of thoroughly cleaned and stored Pyrex tubes interfered with the action of heparin in some unknown manner so that very irregular end-point responses were obtained. Concentration-response clotting curves obtained using freshly cleaned Pyrex glass tubes were similar to those obtained using silicone-coated glass tubes or cellulose nitrate tubes.

IN THE ASSAY method previously described (1) the end point was considered to be that amount of heparin which would just permit the formation of a 50 per cent clot. Since a 50 per cent clot is seldom obtained in any tube, the concentration of heparin corresponding to a 50 per cent clot must be calculated by interpolation from a clotting curve. The degree of clotting in the various tubes has always been estimated visually. It is the purpose of this paper to correlate visually estimated degrees of opacity with opacity measured photoelectrically and with the weights of dried clots. In the course of this work some very marked irregularities were observed when clotting took place in Pyrex glass tubes but were completely eliminated when clotting was allowed to proceed in silicone-coated Pyrex tubes or in cellulose nitrate tubes. A preliminary report of this investigation has been made elsewhere (2).

MATERIALS, CRITERIA, AND PROCEDURES

Opacity determined by inspection is termed visual density. This, representing the degree of coagulation, was estimated in terms of an arbitrary scale, 0 to 4+, with several intermediate values (1).

The transmission of light, here measured photoelectrically, is a reciprocal function of opacity, the latter reflecting the degree of coagulation. Results are reported in terms of per cent transmission of light since this simplified the plotting of curves. However, for convenience, the term optical density is employed. Since optical density is $-\log I/I_0$ the light transmission scale has been inverted to make these curves parallel to those for visual density and clot weight. The instrument accounts for light absorbed (or scattered) by the plasma as well as by the clot itself in contrast to the eye which discounts the plasma color when inspecting for visual density.

In reporting the weights of dried clots it is not presumed that the weights represent pure fibrin. High-speed centrifuging was employed to compact the fibrin clots but it is more than probable that the clots still contained some plasma and/or serum which when dried would add to the weight of the dried fibrin. Certain characteristics of the clot such as size and number of fibrin fibrils, their specific gravity, and their "malleability" must influence the ease of packing during centrifugalization and hence entrained material. It is probable that different concentrations of heparin alter these factors to such an extent that the proportionate amount of packing varies and the ratio of the amount of fibrin present to the entrained impurities is not strictly constant. Nevertheless, it is assumed that the clot weights as determined represent reasonable parallelism to the "true" weight of the fibrin.

* Received Nov. 10, 1948, from the Department of Pharmacology, St. Louis University School of Medicine, St. Louis, Mo. Supported in part by a grant-in-aid from the U. S. Public Health Service.
† The heparin used in this investigation was kindly supplied by Hoffmann-LaRoche, Inc., Nutley, N. J.

Plasma.—Beef and sheep plasmas were employed. The blood when collected was rendered incoagulable with 4 mg. sodium citrate per ml. of blood. After centrifuging for thirty minutes at 2000 r. p. m., the supernatant plasma was siphoned off and frozen until used.

Heparin.—Heparin No. 1 previously reported (3) was dissolved in 0.9% saline in a concentration of 10 γ per ml. for tests on beef plasma and 20 γ per ml. for tests on sheep plasma.

Calcium Chloride.—A concentration of 10 mg. per ml. was employed.

Preparation of Tubes.—Tubes were filled as in the regular assay procedure, that is, in the order: heparin + plasma + saline + calcium. The total volume was either 2.0 ml. or 3.0 ml. The larger volume was required for the electrophotometer used and the proportion of all ingredients was maintained in the same ratio. The plasma volume was 1.0 or 1.5 ml. and the calcium chloride solution 0.2 or 0.3 ml. The tubes were 13 by 100 mm. Unless otherwise stated clotting was allowed to proceed for one hour at room temperature.

Colorimeter Readings.—A Fisher electrophotometer was used with the initial null adjusted to 100% with distilled water as the standard. Most plasmas, beef or sheep, after dilution 1:1, gave readings in the range of 95 ± 2% light transmission. A red filter was used with the rheostat set at *A*.

Clot Weights.—The clots were broken and chopped up in the tubes using a dental spatula. The entire contents were transferred to 13 × 100-mm. cellulose nitrate tubes (CNT), washed several times with distilled water, and placed in a Sorvall Super-Speed centrifuge. The machine was run for one hour at full speed (about 12,000 r. p. m.). The fragments of the clot became compressed into a rubber-like deposit and were removed after three or four rinsings in water. The compressed clot was pressed out firmly on a dry filter paper, placed on a small aluminum weighing boat and dried overnight over calcium chloride. Weighings were made on a Roller-Smith torsion balance. In most tests one hour was allowed for clotting and one hour for centrifuging. The manipulative procedures unfortunately consumed as much as half an hour or more, depending on the number of tubes employed (usually 8 or 16), so that some tubes stood much longer than one hour during the clotting phase.

Besides the above procedure, the clotting reaction was also carried out in the cellulose nitrate tubes during centrifugation. When this was done, the centrifuge was started as quickly as possible (a few seconds) after mixing the ingredients. The tubes were centrifuged one hour.

RESULTS

Section 1. Correlation of Visual Density, Optical Density, and Clot Weight

All three of these measurements were made on each tube. Curves for each of the measurements obtained from a series of tubes containing graded concentrations of heparin have been plotted in Figs. 1 to 4. For graphing, the ordinate scales were adjusted so the three different curves would be approximately superimposed.

Sheep Plasma.—Figure 1 shows the results of allowing the clotting of sheep plasma to proceed in cellulose nitrate tubes for one hour before centrifuging. The concentration of heparin ranged from 0 γ to 22 γ. With no heparin the plasma was considered fully clotted and on the curve for visual density this is represented by 4+. However, by inspection it could be seen that the clot was *less dense* than in the tube containing 16 γ of heparin. The change in optical density in this range is indicated by the curve for light transmission. This effect of heparin has already been discussed (4). As one would expect, the two curves descend substantially together. The curve for optical density does not reach the base line since the unclotted plasma absorbs some

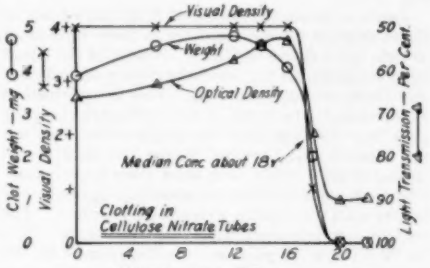

Fig. 1.—Sheep Plasma—SKL (Expt. 79).

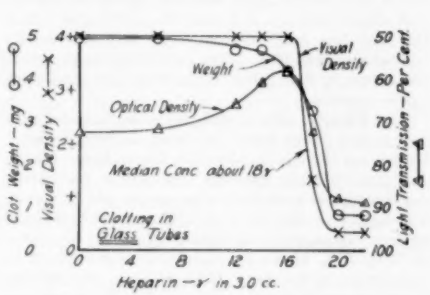

Fig. 2.—Sheep Plasma—SKL (Expt. 79).

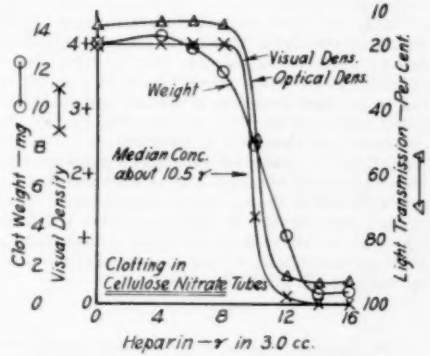

Fig. 3.—Beef Plasma—BKO (Expt. 80).

light.[1] The weight of the clots shows a progressive increase paralleling for some distance the curve of optical density, but the weight begins to decrease *before* the peak in optical density is reached. This break in the weight curve before the peak of the optical density curve was not seen in all experiments. It may reflect an error although the weight curve is smooth.

In Experiment 82 on sheep plasma SIJ (graph not shown), the clot-weight curve remained practically horizontal and descended *with* the curves for visual and optical density (measured in the cellulose nitrate tubes). That is, there was no premature "break" in the weight curve as referred to above. Otherwise, the curves were identical with those of Fig. 1.

Figure 2 shows the results of tests carried out in Pyrex tubes in the same manner as those in cellulose nitrate tubes shown in Fig. 1. Note that the initial tube (no heparin) has somewhat lower optical density (increased transmission). This is explained in Footnote 1. The forms of the curves are the same as in Fig. 1 except that the weight curve does not rise initially. It "breaks" before the peak of the optical density curve as in most other experiments. There was some residual clot present as shown by the failure of all three curves to reach the base line. The median concentration (i.e., CC_{50}) was about 18 γ (or 12 γ per ml. of plasma). In Experiment 82, the results with sheep plasma SIJ in Pyrex tubes were substantially the same except that the weight curve showed a very slight descent up to the "break" which was synchronized with the curves for optical and visual densities; and also, the curves approached the base line more closely. The median dose for both curves was 16 γ. The descending segments of the curves in Experiment 82 were practically identical in position.

Beef Plasma.—Tests with beef plasma in cellulose nitrate and Pyrex tubes are shown in Figs. 3 and 4. In general the curves have the same characteristics as those for sheep plasma. The curve for optical density shows very little rise during the first part. The light was only 10% to 15% whereas with sheep plasma this stage had a light transmission of around 60% or 70% where comparable changes would be proportionally greater. If beef plasma were diluted several times it seems probable that the sensitivity would have increased and the curves approached those for the sheep plasma. The slight increase in the weight of the clot with 4 γ of heparin (Fig. 3) can probably be attributed to technical error. The weight curves "break" before the peak of the optical density (and visual density) just as with sheep plasma. In the Pyrex tubes (Fig. 4) 14 γ and 16 γ fail to produce complete inhibition and with the latter concentration there is an actual increase in clot density as shown by all three curves. Though slight this is probably real since this phenomenon has been repeatedly obtained and is demonstrated in greater detail in the succeeding experiments. The median concentration in the Pyrex tubes is 1.0 to 1.5 γ higher than in the cellulose nitrate tubes. Much greater differences have been found from time to time and will be illustrated later.

In Experiment 85 (not illustrated) in which a different beef plasma, BHI, was used, the results differed slightly. Part of this differing was due to the lower optical density of the final clot. The minimum light transmission in glass tubes was 21% compared to only 10% in Experiment 80 and the curve rose in a manner somewhat similar to the curves for sheep plasma shown in Figs. 1 and 2. The weight curve showed a slight descent before the "break" occurred, the latter being simultaneous with the curves for visual and optical density. In the descending portion the three curves did not agree as well as those in Fig. 4. The median dose was about 13.5 γ. Tests in cellulose nitrate tubes were not run. In Experiment 84 (Fig. 6) the curves for

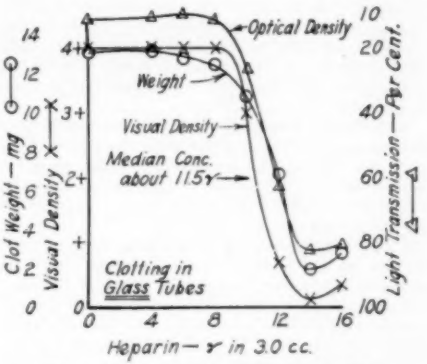

Fig. 4.—Beef Plasma—BKO (Expt. 80).

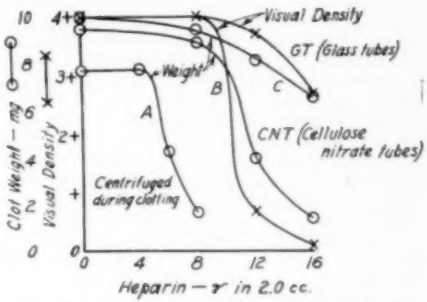

Fig. 5.—Beef Plasma—BKO (Expt. 72).

Pyrex tubes showed poor correlation in the dosage range covered.

Centrifugation during Clot Formation.—When plasma is centrifuged at high speed during the process of clotting, the clot weights obtained are materially less. This is illustrated in Fig. 5 and in Table I. In the graph, comparison is made with clot weights obtained from plasma allowed to clot for an hour before centrifuging. The difference in the initial height of curves *A* and *B* is attributed mainly to greater compaction of the clot which is thrown down by centrifugal force as fast as formed. That is, there is less

[1] The colorimeter readings as per cent light transmission are lower with the cellulose nitrate tubes than with Pyrex tubes because their internal diameter is about 2 mm. greater.

TABLE I.—WEIGHTS OF DRIED CLOTS FROM UNHEPARINIZED PLASMA

Expt. No.	Plasma Sample	Cellulose nitrate tubes Centrifugation During Clotting	Cellulose nitrate tubes Centrifugation After Clotting	Glass tubes Centrifugation After Clotting
72	BKO	7.74	9.45	10.03
73	BKO	8.09	10.10	9.28
				9.95
74	BKO	8.11	...	10.37
		8.08		10.23
				9.75
				10.23
75	BKO	7.70	...	9.77
		7.86	...	8.96
76	BKO	7.87	9.55	9.74
		7.89	8.95	9.51
			9.94	10.29
			10.01 (S)[b]	
80	BKO	7.97	8.89	8.72
	Average:	7.92	9.55	9.76
84	BHI	...	...	6.29
85	BHI	...	...	6.56
77	SKL	2.76	3.15 (S)[b]	3.09
79	SKL	3.35[a]	2.59	3.30
82	SIJ	...	5.70	5.58

[a] Three-minute delay in starting centrifuge.
[b] (S) Silicone-coated tubes.

Experimental Conditions:

Weights: in mg./ml. of original plasma.
BKO, BHI: Beef plasma: SKL, SIJ: Sheep plasma.
Plasma age: varied from 3 minutes to 2¼ hours after thawing.
Clotting time: 1 or 2 hours' duration.
Centrifuging time: 1 hour except in Expt. 72 which was ½ hour.
Clotting temperature: room temperature except for 3 tubes in Expts. 73 and 74 clotted at 37°.
Plasma volume: 1.0 ml. or 1.5 ml. (total volumes 2.0 or 3.0 ml.). Data calculated to 1.0 ml.
The variations in these conditions caused no apparent differences in the clot weights.

entrained plasma or serum present. Table I illustrates this in the clotting of heparin-free plasma. Under the conditions of the test (i.e., Fig. 5.) the heparin was much more effective, the median dose (CC_{50}) being only about half that obtained in the cellulose nitrate tubes where clotting occurred before centrifugation. The time difference could partly explain this since it has been shown (4) that as plasma ages less heparin is required. However the magnitude of the change is not explained on the basis of any data nor is any explanation forthcoming at the moment.

The results shown in Table I, which have already been referred to, were gathered together from a number of experiments in which heparin was used and in which observations on visual density and optical density were made. When tubes were allowed to clot during centrifugation the latter two measurements naturally could not be made. Clotting was allowed to take place during centrifugation to see if there would be any greater compaction than when the centrifuging was done later. This evidently occurs since for plasma BKO the average reduction in clot weight is 17%. This 17% presumably represents the weight of dried entrained serum. But it is probable that the observed weight of the clot is still in excess of the true weight of the fibrin present by more than this 17%.

Section 2. Irregularities Attributable to Surface Effect

Ever since heparin assays were started in 1940 there have been occasional instances where an irregular end point was obtained; that is, clotting (partial or complete) was noted in tubes containing more than the presumed CC_{50} of heparin. These were generally ascribed to technical errors. The end points otherwise have generally been fairly sharp and in line with the curve shown in (1) and with a "spread" much less than indicated by Kuizenga for beef plasma. Recently, however, several series of tests showed very unusual distribution of the erratic end point. Figures 5 to 8 clearly demonstrate the irregularities and will be described in detail.

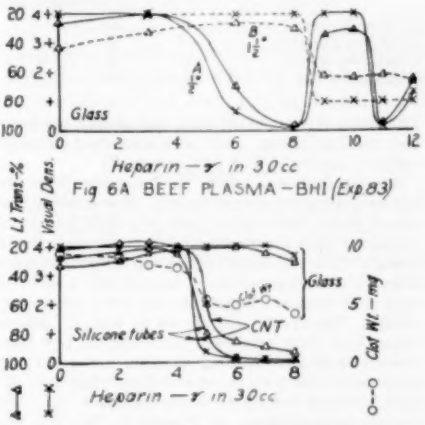

Fig. 6.—Beef Plasma—BHI (Expt. 84).

The effect of the glass surface is shown in Fig. 5 (*C*). Although the plasma was the same as used for the experiments in Figs. 3 and 4, the result, that is, the CC_{50} (by interpolation), was quite different. The inhibiting action of heparin was much less. The estimated CC_{50} is about 18 γ (12 γ/ml. of plasma). This is much higher than the 11.5 γ (7.7 γ/ml. of plasma) found in Fig. 4. The CC_{50}'s obtained in cellulose nitrate tubes were the same in both experiments.

In Fig. 6 the results obtained in silicone-coated and cellulose nitrate tubes show fairly good agreement for both optical and visual densities. The CC_{50} estimated from each curve ranged from 4.5 to 5.0 γ (or 3.0 to 3.3 γ/ml. of plasma). Clot weights were not determined. In glass tubes there was almost complete clotting up through at least 8 γ concentration. The clots were weighed but did not correlate well with the optical and visual densities; actually the weight curve more nearly resembles the optical and visual density curves for clotting in silicone-coated and cellulose nitrate tubes.

Figure 6 (*A*) shows results obtained using only glass tubes. The two curves marked *A* are for measurements of visual and optical density with plasma aged one-half hour after thawing. Since the dosage intervals were wide up to 8 γ the curves as drawn between 3 γ and 8 γ are probably not correct, but should be steeper. At 8 γ the clotting was almost nil but with concentrations of 9 γ and 10 γ there was complete clotting. At 11 γ the curves again approached the base line and at 12 γ rose to nearly a 50% clot. The exact history of these glass tubes prior to use was not recorded except that they were lipless Pyrex tubes and all received the usual acid-dichromate cleaning treatment (1) and were stored for a few days. The clotting was observed closely during the experiment and the relative order of clotting density was noted for the different concentrations of heparin at nine, thirteen, eighteen, and thirty minutes. To a considerable extent this order can be correlated with the curves of Fig. 6 (*A*). The results, omitting the thirteen- and eighteen-minute observations, were:

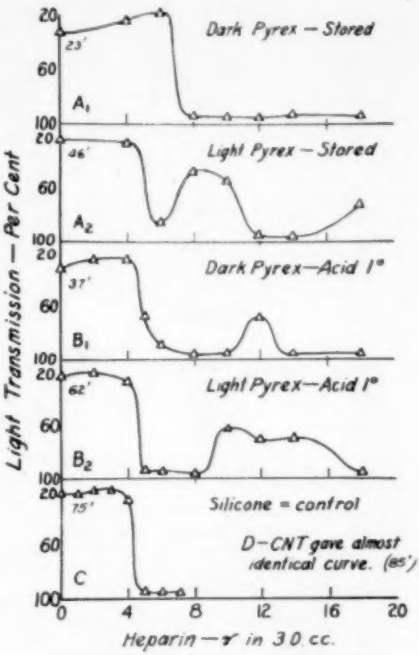

Fig. 7.—Beef Plasma—BHI (Expt. 87).

	Decreasing density →							
At nine minutes:	0	10	9	12	11	3	6	8
At thirty minutes:	0	3	10	9	12	6	8	11
Final, two hours:	0	3	10	9	12	6	11	8 (from curves)
Expected order:	0	3	6	8	9	10	11	12

The tests were repeated with the plasma aged one and one-half hours, again with abnormal although different results (Fig. 6 (*A*), curves *B*).

An attempt was made to determine what influenced the greater irregularity. It was noted that lipped Pyrex tubes had a dark or a light tint when examined closely with side lighting. At first it was thought that the darker tubes were chiefly responsible for the irregularities. Accordingly a series of tests were made with the light and dark tubes separately. The results are shown in Fig. 7. Stored tubes were first employed and then a few were treated with the cleaning solution and used. In these particular tests the stored dark tubes gave the most uniform results as shown in curve A_1. The median concentration of heparin in this test was about 7 γ. The curve leveled off at about the value for the unrecalcified plasma. However, by visual inspection a trace of clot could be seen in the tubes containing 8 γ, 12 γ, and 14 γ of heparin. This series would seem valid except for the comparison with silicone and cellulose nitrate tubes (*C* and *D*) where the median dose was found to be 4.5 γ. The fresh acid treatment for one hour did not alter the response as shown in curves B_1 and B_2.

A few tests were made to determine the influence of alkali and acid on tubes. Light tinted Pyrex tubes were employed. The results are shown in Fig. 8. Silicone tubes served as the control but the amount of plasma available was small so only three concentrations of heparin were employed. In *A*, a dotted line indicates how the curve could be expected to run. In *B*, stored tubes were used. The length of storage was not recorded but it was more than a week. The tubes were kept in dust-free containers. In *C* and *D*, the results with three and one-half hours' soaking in cleaning solution are given. In *C*, the tubes were rinsed in the customary manner with tap and distilled water and finally with redistilled water and then dried in an oven. The tubes used for *D* were similarly treated except that before the final rinsing they were allowed to stand overnight in distilled water. The thought was that possibly the chromium ion (5) might be playing a role and

that overnight soaking would remove the last traces. In both cases the curves are comparable to the silicone curve. This would seem to indicate that tubes should be freshly cleaned with cleaning solution, at least not longer than twenty-four to forty-eight hours before the tests are to be made. If this is the case, one hour is not sufficient soaking time as shown in Fig. 7. In *E* and *F* are shown the effects of rinsing the acid-cleaned tubes with 10% sodium hydroxide. Soaking overnight in water after the alkali treatment did not improve the results. Presumably some alkali adhered to the glass permanently.

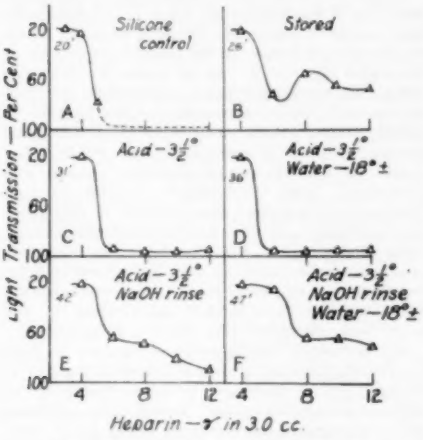

Fig. 8.—Beef Plasma—BHI (Expt. 88).

A few other tests were made on the effect of acid or alkali. In Experiment 85 a few Pyrex tubes freshly cleaned in the acid-dichromate cleaning solution were used. With beef plasma BHI concentrations of heparin of 8 γ, 10 γ, 12 γ, and 15 γ gave just traces of clot in all tubes. In stored tubes with the same doses, the clots were 4+, 4+, 3+ and +(−), respectively. In Experiment 86, plasma BHI was used. Five minutes' soaking in acid cleaning solution resulted in the same erratic response as in stored tubes.

In Experiment 89, using plasma BKO, stored tubes gave erratic responses while results using silicone tubes were normal (cf. Fig. 7, A_2 and *C*). Tubes soaked twenty-four hours in cleaning solution gave curves similar to the silicone series. Again stored tubes gave erratic responses with very flattened descending curves for visual and optical density.

Experiment 90 was conducted using sheep plasma SN. The erratic behavior was considerably less than that noted for beef plasma. In stored tubes a slight film of clot adhered to the walls of two tubes whereas with the same heparin concentrations in freshly cleaned tubes (forty-eight hours in acid; rinsed the day used) there was no film with these same concentrations. One series (Experiment 90-D) was run with narrower concentration intervals using stored tubes. The curves for both optical and visual density were flatter than usually obtained for sheep plasma. The heparin concentration spread for the descending portion of the two curves was about 60%, that is, the first tube with no clot had 60% higher heparin concentration than the last tube showing complete clotting.

In still other cases in experiments where regular assays were being conducted, sheep plasma showed a tendency to form a film of clot on the tube walls with concentrations of heparin well beyond the end point and to a degree approaching that seen using beef plasma (3). However, in the many sheep plasmas tested (and usually using "stored" tubes) this result has been the exception rather than the rule.

In the light of present data it may be concluded that if Pyrex tubes are soaked in strong, sulfuric acid-dichromate cleaning solution overnight, and within twenty-four to forty-eight hours of use, the results are more likely to resemble those obtained in silicone or cellulose nitrate tubes when either beef or sheep plasma is employed.

CONCLUSIONS

1. In most tests there was close correlation between visual density, optical density, and clot weight in the descending segments of the curves. This range includes the criterion for the end point, the 50 per cent clotting concentration (CC_{50}). Validity of the end point obtained by visual inspection in heparin assays is thus established.

2. Serious irregularities have been encountered in the clotting of recalcified heparinized beef plasma and to a much lesser extent in sheep plasma when clotting took place in Pyrex tubes that had been stored for some time. The use of silicone-coated tubes or cellulose nitrate tubes completely eliminated the irregularity.

3. The present observations imply that Pyrex glass tubes should be freshly cleaned with sulfuric acid-dichromate cleaning solution within a day or so of their use and with at least several hours' soaking before thorough rinsing with water.

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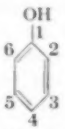
Comparative Toxicity of Certain *t*-Butyl Substituted Cresols and Xylenols*

By W. A. McOMIE, HAMILTON H. ANDERSON, and F. M. ESTESS

Of the compounds studied, 2-*t*-butyl 4-methylphenol, 2-*t*-butyl 5-methylphenol, 2,4-di-*t*-butyl 5-methylphenol, 2,6-di-*t*-butyl-4-methylphenol and 3,5-dimethylphenol have a lower degree of acute toxicity than phenol itself. However, 2,4-dimethyl-6-*t*-butylphenol is unique in its toxicity to the rabbit, being as lethal by simple application to the intact skin as by the oral route of administration. This is apparently a species susceptibility since rats and guinea pigs are less susceptible to the compound by both the oral and the skin route. It is concluded that the dialkyl phenols are more irritant locally than the trialkyl phenols studied.

THE COMPOUNDS of chief interest[1] in this study are important as oxidation inhibitors. They are all derivatives of phenol and for sake of brevity will be designated by the position of the alkyl groups on the ring relative to the phenolic hydroxyl group according to the following scheme.



2-*t*-Butyl 4-methylphenol (2B4M)
2-*t*-Butyl 5-methylphenol (2B5M)
2,4-Di-*t*-butyl 5-methylphenol (24B5M)
2,6-Di-*t*-butyl 4-methylphenol (26B4M)
6-*t*-Butyl 2,4-dimethylphenol (24M6B)
3,5-Dimethylphenol (35M)

Parallel experiments were conducted in certain instances with phenol, carvacrol, and thymol.

All of the compounds contained less than 1 per cent of impurities. Carvacrol, 2B5M and 24M-6B were liquids at 25°. The others were solids. The physical properties of several compounds were reviewed by Pardee and Weinrich (1).

The purpose of this study was to determine the relative local and systemic toxicities of the compounds and to estimate their relative hazard to man.

EXPERIMENTAL

Intragastric Administration to Mice.—Cottonseed oil solutions (1–4%) of the phenols were administered to previously fasted (sixteen to twenty-four hours) stock white, male, mice (weighing from 15 to 25 Gm. each) by means of a blunt-tipped 18-gauge needle attached to a syringe. Volumes given varied from 0.2–0.8 cc. (Table I).

After intragastric administration of cottonseed oil solutions of all compounds other than phenol, the mice reacted in essentially the same manner. There usually was depression to the point of prostration at the higher dose levels. At the lower dose levels no effect other than depression was observed. This was the general observation after administration of 2B4M, 2B5M, 24B5M, 24M6B, 26B4M, 35M, and thymol. Phenol in doses as low as 280 mg./Kg. caused all mice to exhibit muscular tremors and generalized body twitchings. Deichmann and Witherup (3) described these tremors or twitchings in rats, rabbits, and cats after phenol administration. These authors also found that *o*-, *m*-, and *p*-cresols cause these twitchings but were less severe with the cresols than with phenol itself. From our results it would appear that further substitution in the cresol molecule causes this effect to disappear.

The pathologic effects produced in mice by these compounds consisted of irritant action on the gastroenteric tract as evidenced by hemorrhages in the region of the small intestine. Severe edema and congestion of the lungs were usually found grossly after both acute and delayed deaths. There was no microscopic evidence of myocardial damage. Delayed deaths occurred after exposures to some of these compounds. An observation period of ten days was chosen arbitrarily. Some animals died as long as ten days after administration of the compounds. Phenol, 35M, and thymol were exceptions to this delayed-death effect. In no instance after application of these three compounds did death occur after forty-eight hours. The tri-substituted compounds, however, were particularly noteworthy in this delayed death effect. In the case of 24M6B and 26B4M over 50% of the deaths occurred ninety-six hours after application of the compounds. Livingston (4) found that delayed deaths (five days and more after administration) took place with carvacrol and thymol after intragastric administration to rabbits.

Administration to the Skin of Rabbits.—The liquid compounds (2B5M, 24M6B, and carvacrol) were placed in rubber cuffs and the solid compounds on gauze held in place by a flexible wire screen according to the method of Draize, *et al.* (5). In the case of the liquid compounds the exposures were for six hours. The severe local effects of 2B5M and carvacrol were evident after this time and 24M6B was lethal. All of the solids were allowed to remain on the skin for twenty-four hours (Table II). The compounds were also applied in ether by flowing the ether solution on to the shaved back of the rabbit (Table III).

From the above results the following generalizations were made concerning these compounds after administration topically to rabbits:

* Received September 20, 1948, from the Division of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco 22, Calif.
[1] Supplied by the Shell Development Co., Emeryville, Calif., who aided in the support of this study.

TABLE I.—ACUTE INTRAGASTRIC TOXICITY OF ALKYL PHENOLS IN COTTONSEED OIL SOLUTION TO MICE

Dose, Mg./Kg.	Mortality Ratio (10 Days' Observation)							
	2B4M	2B5M	24B5M	24M6B	26B4M	35M	Thymol	Phenol
280	0/10	...	...	3/10	...	0/10	...	0/5
420	1/10	...	...	4/5	1/10	1/10	...	0/5
620	7/10	2/5	1/10	5/10	4/10	4/10	0/5	4/5
940	5/10	1/10	2/10	4/5	4/10	9/10	0/5	5/5
1400	...	6/10	3/10	...	6/10	...	3/10	...
2100	...	5/5	9/10	...	...	...	3/5	...
LD_{50}[a]	700	1080	1420	530	1040	620	1800	540[b]
Standard Error LD_{50}	±35	±93	±108	±36	±204	±52	±224	...

[a] Graphic method of Miller and Tainter (2) was used to obtain the approximate LD_{50}'s and their standard errors.
[b] Estimate.

TABLE II.—CUFF EXPOSURES OF RABBITS TO ALKYL PHENOLS

Compound	Dose[a]	Duration of Exposure, Hr.	Effects Noted
Carvacrol	2.7	6	Immediate irritation, becoming erythematous. Leather-like appearance within 4 hr. Complete necrosis and eventual sloughing and scar formation. Died 72 hr. after exposure.
2B4M	2.2	24	Similar local effects as with carvacrol. Died 72 hr. after exposure.
2B5M	2.9	6	Similar local effects as with carvacrol. No apparent systemic effect other than that due to severe burn of the skin. Survived.
2B5M	3.9	6	Similar picture as above. Survived.
35M	2.6	24	Similar effect as with 2B5M. Survived.
24B5M	4.5	24	Only slight irritation of skin (mild erythema without subsequent necrosis). No apparent systemic effect.
26B4M	3.3	24	No evidence of skin irritation. No apparent systemic effects.
24M6B	2.9	6	Died within 6 hr. from beginning of exposure. No local erythema or edema. Lung grossly edematous and congested.
24M6B	3.9	6	Same effect as above. Both animals had sufficient lung edema to have caused death.

[a] Dose is given in cc./Kg. for the liquid compounds (2B5M, carvacrol, and 24M6B) and in Gm./Kg. for the solid compounds. One rabbit was used at each dose level.

TABLE III.—APPLICATION OF ETHER SOLUTIONS OF ALKYL PHENOLS TO THE SKIN OF THE RABBIT

Compound	Dose, Gm./Kg.	Effects Noted
Phenol[a]	0.42	Skin turned parchment-like within 24 hr. No apparent systemic effect. Complete necrosis of superficial layers after 10 days. Survived.
Thymol	0.42	Similar picture to that of phenol.
35M	0.42	Similar picture to that of phenol.
2B4M	0.42	Similar picture to that of phenol.
2B5M[b]	0.42	Similar picture to that of phenol.
24B5M	0.42	Some erythema and superficial sloughing but no severe tanning effect as seen with phenol.
26B4M	0.42	Similar picture to that of 24B5M.
24M6B	0.08	Died 36 hr. after application. No local effect. Edematous lungs found upon gross autopsy.

[a] Liquefied phenol, U. S. P. XIII, was used.
[b] Given undiluted.

1. 2B4M, 2B5M, 35M, and carvacrol are severely irritant and resemble phenol in their coagulative effect on the skin. Carvacrol and 2B4M may cause fatalities through exposure of relatively large areas of the skin. This may be due to secondary effects from the severe chemical burn rather than from systemic absorption of the compounds themselves.

2. Although 24M6B is only mildly irritant it is readily absorbed from the skin and causes death through systemic action, rather than indirectly from skin injury.

3. The two solid tri-substituted alkylated phenols (24B5M and 26B4M) are the least irritant, and evidently little is absorbed from the solid form.

4. None of the other compounds had any apparent systemic effect after topical applications of a dose 5 times greater than the lethal dose of 24M6B (see Table V).

Effect of Alkyl Phenols on the Eyes of Rabbits.—Table IV summarizes the results of exposure to the eyes of rabbits. In each case 0.03 cc. of the solution was applied directly to the cornea.

The irritation to the rabbit's eye of these compounds places them in similar categories as shown previously for their action on the skin. Thymol, 35M, 2B4M, and 2B5M are all extremely irritant; 24B5M and 24M6B are less irritant but still dangerously irritant to mucous surfaces. 26B4M, due to

its lack of solubility, could not be given in a water-miscible solvent. Its lack of irritation in diallyl phthalate (6) might indicate that it had a much greater affinity for the relatively bland medium in which it was dissolved rather than for the corneal or conjunctival membrane. Allowing for this inability to demonstrate irritation in the case of 26B4M, it is apparent that all the compounds are injurious to the eye but are not particularly more so than thymol.

guinea pigs and rats by simple application without rubbing. The lethal doses obtained by this method are shown in Table V.

In rabbits there was a remarkable paralleling of toxicity by the oral and skin routes of administration. However, the rat and the guinea pig are not only less susceptible than the rabbit to intragastric administration of the compound but also show less likelihood of toxic effects through skin absorption.

TABLE IV.—APPLICATION OF 0.03 CC. ALKYL PHENOLS TO THE RABBIT CORNEA

Compound	Concentration	Result[a] in 1 Hr.	Result[a] in 24 Hr.	Result[a] in 72 Hr.	Fluorescein[b] Test, 24 Hr.
2B4M	40% in ethylene glycol	31	80[c]	80[c]	[d]
2B5M	Undiluted	32	78	80[c]	100
24B5M	40% in ethylene glycol	13	9	2	90
24M6B	Undiluted	0	43	53	90
26B4M	40% in diallyl phthalate	0	0	0	0
35M	40% in ethylene glycol	31	80[c]	80[c]	90
Thymol (control)	40% in ethylene glycol	32	80[c]	80[c]	100

[a] Score of Draize, *et al.* (5), used (1944); the larger the number, the greater the injury to the mucous membranes.
[b] Numbers under fluorescein refer to percentage staining of corneal surface with fluorescein and indicate extent of damage to corneal epithelium.
[c] After 24 hr. the eye was closed so completely that injury could not be evaluated by the scoring method. The maximum score possible (110) was approached in the case of 2B4M, 2B5M, 35M, and Thymol. Permanent clouding of cornea resulted in case of 24M6B.
[d] Eye was completely closed and could not be opened to instill the fluorescein.

TABLE V.—COMPARATIVE LETHAL DOSES OF 24M6B TO RABBITS, RATS, AND GUINEA PIGS BY THE ORAL AND SKIN ROUTES OF ADMINISTRATION

	Oral			Skin		
	Rabbits	Rats	Guinea Pigs	Rabbits	Rats	Guinea Pigs
Lethal dose,[a] cc./Kg.	0.055	1.4	0.42	0.055	10.7[b]	7.1

[a] Lethal dose as defined by Deichmann and LeBlanc (7) as first lethal dose in the series.
[b] No deaths occurred. This was highest amount given.

Comparative Toxicity of 24M6B by Skin and Oral Routes of Administration.—The results of skin application to rabbits indicated that 24M6B had a systemic toxicity considerably above any of the other compounds when applied by this route. In addition it was not as irritant as some of the other compounds possibly rendering its hazard even greater since lack of irritation would not provide warning and could lead the person exposed to a false sense of security. Accordingly, a study of the comparative toxicity to rabbits, rats, and guinea pigs by the oral and skin routes of administration was made. The method of Deichmann and LeBlanc (7) was used for determining the approximate lethal doses by these routes. This method consists briefly of giving a single animal a dose differing by 50% from the preceding dose given to another animal. The approximate lethal dose by their definition is the lowest dose of the series that kills. These authors showed that for a group of compounds this approximate lethal dose varied from the LD_{50} by about ±30%. Employing this method (with the exception that in some cases more animals were employed at each dose in the lethal range), the following results were obtained. Compounds were given orally either undiluted or dissolved in propylene glycol. Topical applications were made to the previously clipped area of the backs of guinea pigs and rabbits and to the abdominal surface of rats. The undiluted compound or ether solutions of each (when small amounts were applied) were given to The rat, in particular, is quite resistant by both the oral and the skin routes.

The signs of toxicity in rabbits were, first, depression, then dyspnea accompanied by râles and frothing at the nostrils. Convulsions ensued terminally probably due to asphyxia as the result of profound lung hemorrhage and edema, easily seen grossly at autopsy.

Signs of poisoning in rats and guinea pigs were essentially the same as in rabbits. The lung changes were not as severe as in rabbits even with lethal doses. The stomach wall and intestines were highly inflamed in both guinea pigs and rabbits after oral administration of the undiluted compound or as a 10% solution in propylene glycol. The evidence on species difference in susceptibility to 24M6B seems unmistakable. An indication that other compounds were not as toxic as 24M6B to the rabbit was seen when single rabbits survived intragastric doses of 0.62 Gm./Kg. of 26B4M and 0.94 Gm./Kg. of 2B5M, respectively, without any apparent effect. Both of these amounts are more than 10 times the intragastric LD_{50} for rabbits of 24M6B.

DISCUSSION

Aside from the previous communication on the toxicity of 24M6B by Treon and Cyama (8) no other data concerning the toxicity of these compounds have appeared in the literature. Related alkyl

phenols have been investigated by Lamson, *et al.* (9, 10) and studies have been made comparing the toxicity of thymol and carvacrol by Livingston (4) and Caujolle and Franck (11). Deichmann and Witherup (3) have compared the toxicity of phenol and the *o*, *m*-, and *p*-cresols.

The substituted cresols and xylenols studied, with the exception of 24M6B, in the rabbit, are more comparable in toxicity to thymol than to phenol itself. It would seem, in view of the widespread use of thymol in pharmaceutical products, that these compounds should present no particular hazard if used with caution.

The unique toxicity of 24M6B in the rabbit is of interest because its lethal dose by the skin and oral routes is nearly identical. The mechanism of this toxicity and the reason for its ease of penetration through the intact rabbit skin are as yet unexplained. This compound should be treated with utmost caution in view of its extreme toxic effects in the rabbit.

SUMMARY

1. The range of acute intragastric LD_{50} for mice of cottonseed oil solutions of certain *t*-butyl substituted compounds was from 530–1800 mg./Kg. The relative order of toxicity by this route from most toxic to least toxic was 6-*t*-butyl 2,4-dimethylphenol, phenol, 3,5-dimethylphenol, 2 *t*-butyl 4-methylphenol, 2,6-di-*t*-butyl 4-methylphenol, 2-*t*-butyl 5-methylphenol, 2,4-di-*t*-butyl 5-methylphenol, and thymol. The compounds may be considered as of intermediate toxicity between phenol and thymol in so far as the intragastric route for mice is concerned.

2. Carvacrol, thymol, phenol, 2-*t*-butyl 4-methylphenol, 2-*t*-butyl 5-methylphenol, and 3,5-dimethylphenol were severely irritant to the skin of rabbits upon prolonged exposure. The trisubstituted compounds (2,6-di-*t*-butyl 4-methylphenol, 2,4-di-*t*-butyl 5-methylphenol, and 6-*t*-butyl-2,4-dimethylphenol) while irritant are not in the same category as the dialkyl derivatives or phenol itself.

3. The compound 6-*t*-butyl 2,4-dimethylphenol, though only mildly irritant to the skin of rabbits, caused death in this species with as small a dose as 52 mg./Kg. applied to the skin. The LD_{50} of this compound to rabbits is approximately the same by either the oral or the skin routes. Rats and guinea pigs, however, were less susceptible to its toxic action after intragastric administration and much less susceptible after percutaneous application.

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Microchemical Identification of Amidone*

By ROBERT C. WATSON† and MAX I. BOWMAN‡, §

Amidone may be identified microchemically through formation of crystals with certain reagents. Tests with palladium chloride, potassium ferrocyanide, and bromine water are described and a table of the crystal-forming action of these reagents on the salts of 34 alkaloids and synthetic drugs is presented.

SINCE the discovery of amidone (6-dimethylamino-4,4-diphenyl-3-heptanone)[1] in the 1930's and its subsequent introduction into medicine as an analgesic, considerable interest has been shown toward it by the field of clinical medicine (1).

Because of its addiction liability and morphine-like action, amidone falls within the purview of the U. S. Bureau of Narcotics as a restricted narcotic drug. It is, therefore, desirable to have a relatively simple and rapid method of identifying samples which are seized from illicit channels.

Amidone may be readily identified microchemically. Its reaction with 197 reagents, including many of the common so-called "alkaloidal re-

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§ The writers wish to acknowledge their appreciation to Raymond A. Bevins, Identification Section, Alcohol Tax Unit, Louisville, Ky., for assistance in preparing the photomicrographic plates.
[1] Also known in the drug field as dolophine or methadon.

agents" was examined. Of these, 43 gave crystals, the remainder giving only amorphous precipitates or none at all. Only 3 reagents were selected as giving well-defined crystals of characteristic shapes suitable for identification purposes, the others being rejected on the basis of atypical crystals, or because of similar crystal formation with many common alkaloids.

TABLE I.—REACTIONS OF AMIDONE GIVING CRYSTALLINE PRECIPITATES[a]

Reagent	Type of Crystals
Ammonium acetate	Rectangular plates (R)
Ammonium carbonate	Bundles and rosettes of plates
Ammonium hydroxide	Dense masses of rectangular plates
Ammonium iodide	Dense rosettes of feather-like crystals
Ammoniacal nickel acetate (2)	Clumps of indistinct crystals
Ammonium oxalate	Needles and rods (R)
Ammonium sulfide	Rectangular plates (R)
Ammoniacal silver nitrate (2)	Masses of poorly defined crystals
Barium acetate	Rectangular plates (R)
Barium chloride	Mostly small plates (R)
Barium hydroxide (sat. soln.)	Fine root-like branching crystals (R)
Bromine water (sat. soln.)	Flat plates with notched ends, later rosettes of plates
Cadmium iodide	Bundles of small, branching, rod-like crystals
Calcium chloride	Rectangular plates (R)
Hydrobromic acid (conc.)	Mostly diamond-shaped plates and tablets
Hydriodic acid (47%)	Bundles and rosettes of plates
Iodine water (sat. soln.)	Small "X"-shaped plates
Iodine-Potassium iodide	Rectangular and notched plates
Mercuric chloride	Bundles of rod-like plates
Mercuric nitrate (sat. soln.)	Bundles of flat rods
Magnesia mixture (2)	A few rectangular plates and tablets (R)
Nickel acetate	Some rectangular plates (R)
Palladium chloride (in dil. HCl)	Twinned spearhead-like crystals, later fern-like branches
Potassium acetate	Tablets and plates (R)
Potassium-Bismuth iodide	A few rectangular plates (R)
Potassium bitartrate	Rods and tablets (R)
Potassium chromate	Irregular rosettes of plates
Potassium ferrocyanide	Rods and rosettes of rod-like crystals
Potassium iodide	Dense rosettes of fern-like crystals
Potassium nitrite	Masses of overlapping plates
Potassium oxalate	Mostly rectangular plates and fragments (R)
Saccharin	Overlapping plate formations
Silver iodide-Potassium iodide (1:15)	A few rosettes of irregular plates
Sodium acetate	Irregular plates
Sodium bicarbonate	Irregular chain formation of plates
Sodium borate	Network of indistinct crystals
Sodium carbonate	Clumps of indistinct crystals
Sodium fluoride	Mostly hexagonal tablets (R)
Sodium iodide	Dense rosettes of branching needles
Sodium phosphate (dibasic)	Rectangular plates (R)
Sodium silicate (sat. soln.)	A few small square plates (R)
Sodium sulfite	Irregular plates
Sodium tungstate	Masses of indistinct crystals

[a] Crystal types marked (R) appear, on preliminary examination, to be reagent crystals thrown from solution.

EXPERIMENTAL

The tests were made in the usual manner by placing the reagent drop on a microscopic slide adjacent to the sample drop and allowing the two drops to flow together. The slide was allowed to stand for a short time and then was examined under a microscope (magnification ×100) for crystal formation.

Photomicrographs were made of characteristic crystals using a 4 x 5-in. Speed Graphic plate camera. The lens was removed and the microscope draw-tube extended into the camera bellows, the image being brought to sharp focus on the ground glass screen by rotating the micrometer head of the microscope.

Aqueous amidone hydrochloride solutions of 1:50, 1:200, 1:500, and 1:1000 concentrations were tested. In no instances were the slides scratched to hasten crystal formation. The use of cover glasses was avoided since they tended to cause the formation of distorted crystals.

PREPARATION OF REAGENTS

Palladium Chloride.—(Approximately 1% in dilute HCl.) Make up 1 Gm. $PdCl_2 \cdot 2H_2O$ to 100 ml. with dilute HCl (35 ml. HCl, *s. g.* 1.19, *q. s.* 100 ml. with distilled water); warm gently until solution is complete.

Potassium Ferrocyanide.—(Approximately 5% aqueous solution.) Make up 5 Gm. $K_4Fe(CN)_6$

$3H_2O$ to 100 ml. with distilled water (3). This reagent should be made up fresh each day since it slowly decomposes on standing.

Bromine Water.—(Dilute solution.) Make up 3.5 ml. saturated bromine water to 100 ml. with distilled water. This reagent should be made up frequently.

Amidone reacted with approximately 1% aqueous solutions of 43 reagents to give crystalline precipitates with the results shown in Table I.

Only amorphous precipitates were obtained with ammonium molybdate, antimony trichloride, arsenic chloride, barium nitrate, bismuth nitrate, cadmium chloride, calcium hydroxide, chromic acid, chromium nitrate, cobalt nitrate, cobalt thiocyanate (blue ppt.), cupric nitrate, 2,4-dinitro-phenylhydrazine, ferric nitrate, Frochde's Reagent (3) (white ppt.), gold chloride, iridium chloride, Kraut's Reagent (3), Lanthanum nitrate, lithium carbonate, lithium chloride, lithium nitrate, Marme's Reagent

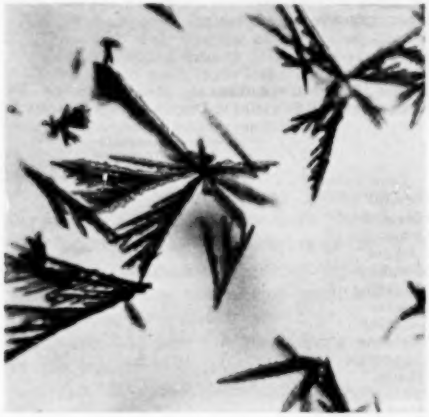

Fig. 1.—Amidone 1:50—palladium chloride crystals.

(3) (grayish white ppt.), Mayer's Reagent (3) (white ppt.), Millon's Reagent (3) (white ppt.), molybdic acid, nickel nitrate, picric acid, picrolonic acid, Phenylhydrazine-HCl, phosphomolybdic acid, phospho-tungstic acid, platinum chloride, potassium acid phthalate, potassium carbonate, potassium chloride, potassium cyanide, potassium dichromate, potassium ferrocyanide, potassium hydroxide, potassium permanganate, potassium phosphate, potassium sulfide, potassium-zinc-iodide, rhodium chloride, ruthenium chloride, silico-tungstic acid, silver acetate, silver sulfate, sodium-benzene-sulfonate, sodium bichromate, sodium-cobalt-nitrite, sodium nitro-prusside, sodium peroxide, sodium phospho-molybdate, sodium sulfide, sodium thiocyanate, stannous chloride, tetra-chloro-phthalic acid, Wagner's Reagent (3), zinc-chloro-iodide.

Crystals Best Suited for Identification.—In making the tests one drop each of a $^1/_{50}$, $^1/_{200}$, $^1/_{400}$, and $^1/_{1000}$ aqueous solution of amidone hydrochloride was placed on a microscopic slide together with an equal drop of the reagent. The slide was allowed to stand for a short time until crystal formation occurred and then microscopic examination was made under a magnification of 100×.

Palladium Chloride.—These crystals form almost at once from an amorphous precipitate in the $^1/_{50}$ solution (Fig. 1), and somewhat slower in the $^1/_{200}$ solution (Fig. 2). In greater dilutions the crystals were very slow in forming and resembled double-headed flint spearheads (Fig. 3). These crystals

Fig. 2.—Amidone 1:200—palladium chloride crystals.

Fig. 3.—Amidone 1:500—palladium chloride crystals.

show brilliantly under polarized light. They grow to considerable size, forming rosettes of brown fern-like plates or fragments. One notable feature is the fact that the rod-like crystal growth occurs on one side only of each arm and at an angle of approximately 30° from the main axis. This was found to be the most satisfactory crystalline test.

Potassium Ferrocyanide.—Crystals form at once from a clear solution in the $^1/_{50}$ concentration (Fig. 4). At first these crystals appear as bundles of needle-like rods curving outward at the ends, having somewhat the appearance of sheaves of grain. Later rosettes of plates were formed. Crystals were not formed in the more dilute solutions. The three dimensional growth of these crystals may best be observed under polarized light.

Bromine Water.—Many small crystals formed at once in all solutions, but those in the $^1/_{200}$, $^1/_{500}$, and $^1/_{1000}$ solutions were best for identification purposes (Fig. 5). This test was improved by adding a drop of dilute HCl to the amidone solution and stirring before adding the bromine water. The crystals at first appear to be single plates having notched ends in such a manner as to resemble the letter "N," when slightly out of focus. Later they develop into rosettes of rod-like plates.

Fig. 4.—Amidone 1:50—potassium ferrocyanide crystals.

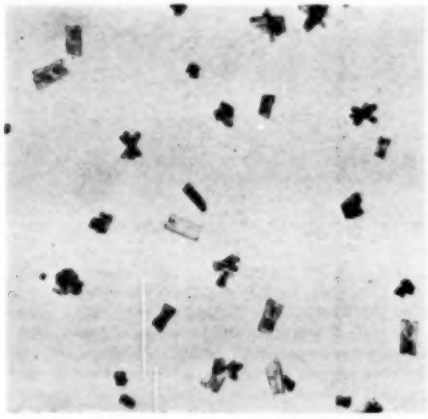

Fig. 5.—Amidone 1:200—bromine water crystals.

Crystal Formation with Other Drugs.—The selected reagents were tested for crystal formation with 34 alkaloidal salts and synthetic drugs. Eight crystalline and 13 amorphous precipitates were obtained as indicated by Table II. These crystals have distinctive shapes not easily confused with those formed with amidone.

CONCLUSIONS

Amidone may positively be identified by microscopic examination of crystals formed with palladium chloride, potassium ferrocyanide and bromine water. These reagents give relatively few crystalline precipitates with other common alkaloids and synthetic drugs.

The search for crystal-forming reagents outlined by the authors was not an exhaustive one. It seems likely, therefore, that characteristic crystals suitable for identification purposes might be formed with other reagents.

TABLE II.—MICROCHEMICAL REACTIONS OF 34 ALKALOIDS AND SYNTHETIC DRUGS WITH 3 REAGENTS

	Reagents		
Drug Tested	Palladium Chloride	Potassium Ferrocyanide	Bromine Water
Dicodid	...	...	...
Dilaudid	...	...	...
Demerol	...	...	...
Cocaine	...[a]	...[b]	...
Morphine	...	A	...
Narcotine	A	...	A
Codeine	...	...	A
Narceine	...[a]	C[a]	...
Thebaine	A	...	...
Papaverine	A	A	A
Dionin	...	...	...
Pantopon	...	...	...
Heroin	...	...	...
Atropine	...	...	...
Caffeine	C	...	...
Brucine	C	...	...
Strychnine	C	C	...
Saccharin	...	...	...
Stovaine	...	C	A
Quinine	...	A	A
Novocaine	...	...	...[c]
Alypin	...	...	A
Phenacetin	...	...	...
Sulfon-Methane	...	...	...
Na-Phenobarbital	C	...	...
Nembutal	C	...	...
Seconal	A	...	...
Ephedrine-Amytal	...	...	...
Digitalin	...	...	...
Nitroglycerin	...	...	...
Scopolamine	...	...	...
Strophanthin	...	...	...
Ergotrate	...	...	...
Nicotine	...	...	...

(A—amorphous precipitates; C—crystalline precipitates)

[a] Disagreement with the findings of Stephenson (4) previously reported. The concentrations used above in most instances differed from those used by Stephenson.

[b] Rosenthal (5) obtained six-sided crystals with cocaine using ferrocyanide as a reagent.

[c] Saturated bromine water gives bundles of crystals with novocaine.

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Spectrophotometric Assay Method for Pyranisamine Maleate*

By LUISE T. ANDERSON, W. C. GAKENHEIMER, CHARLES ROSENBLUM, and E. H. SMITH

A spectrophotometric assay method for pyranisamine maleate, the nonproprietary name of a new antihistaminic compound being marketed under the trade-mark "Neo-Antergan" maleate, in various pharmaceutical preparations is described together with a discussion of the ultraviolet absorption characteristics of this compound. Tabulated results indicate that assay deviations from the expected composition are in most cases less than 5 per cent. The constancy of the absorption spectra of pyranisamine maleate in these preparations indicates the stability of the antihistaminic in these preparations.

PYRANISAMINE maleate is the nonproprietary name of a new antihistaminic compound being marketed under the trade-mark "Neo-Antergan" maleate. The incorporation of this compound into pharmaceutical preparations such as tablets, ointments, elixirs, and parenteral solutions is being investigated.

The salt is obtained by neutralizing one mole of the free base N-(α-pyridyl)-N-(*p*-methoxybenzyl)-N′,N′-dimethylethylenediamine with one mole of maleic acid. Being an aromatic substituted derivative of ethylenediamine, pyranisamine exhibits an ultraviolet absorption spectrum sufficiently intense and characteristic to distinguish it from substances commonly present in pharmaceutical preparations, a fact which serves to simplify the assay of such preparations. This was ascertained by studying the absorption spectral characteristics of the maleate in aqueous solutions over a wide *p*H range, in 20 per cent ethyl alcohol and in a mixture of absolute ethyl alcohol and petroleum ether. The latter solvents were included in the investigation because they facilitate the dissolution of certain types of pharmaceutical preparations. The contribution of maleic acid to the absorption spectrum of the salt was also studied in aqueous solutions. The spectrophotometric study will be described below, together with illustrative applications to the assay of typical pharmaceutical preparations.

* Received March 3, 1949, from the Research Laboratories, Merck & Co., Inc., Rahway, N. J.

SPECTROPHOTOMETRIC STUDY

Materials.—Four samples of pyranisamine maleate were used to determine its absorption spectrum. Melting points and carbon, hydrogen, and nitrogen data are listed in Table I. Monsanto maleic anhydride was used in the preparation of maleic acid solutions. The ethyl alcohol and petroleum ether (b. p. 50–60°) employed as solvents in certain measurements were of reagent grade, as were the materials required for the preparation of certain of the buffers (*p*H's 4.4, 7, and 11). Other buffers were of the "Parstains" phosphate type commercially available.

TABLE I.—PROPERTIES OF PYRANISAMINE MALEATE SAMPLES

Sample	M. P., °C.	C	H	N
A	101.5–103	63.03	6.78	10.46
B	101	62.93	6.42	10.69
C	101	62.85	7.08	10.74
D	101–102	62.41	6.65	10.76
Theoretical	...	62.83	6.78	10.87

Measurements.—Absorption spectra (optical density vs. wave length) of solutions were measured in 1-cm. quartz cells with a Beckmann, Model DU. Quartz Spectrophotometer, the solvents themselves being used as reference liquids. Solutions were prepared for observation by dissolving 2 mg. of the maleate (accurately weighed on a microbalance) in 100 ml. of solvent, which was usually distilled water or an aqueous buffer, though 20% ethyl alcohol and a 50-50 mixture of absolute ethyl alcohol and petroleum ether were also investigated. Spectra in aqueous solutions were determined at *p*H's ≃4 ($10^{-4}N$ HCl), 4.4 (acetate buffer), 5 ("Parstains" phosphate buffer), 5.5 ("Parstains" buffer), 6.0

("Parstains" buffer), 6.5 ("Parstains" buffer), 7 (monobasic phosphate-alkali buffer) and 11 (tetraborate-alkali buffer). All *p*H's were measured with a Cambridge Research Model *p*H Meter after solution of the pyranisamine maleate. The spectral characteristics of maleic acid itself were determined by dissolving ≃2 mg. of anhydride (microbalance) in 100 ml. of water, dilute acid, or alkali to yield unbuffered solutions of *p*H 3.5, 5.3, 7.1, 9.5, and 11.2.

Ultraviolet Absorption Spectra.—The spectrum of pyranisamine maleate changes with acidity and with solvent. In aqueous solution at *p*H <5.5, it consists of two bands located at 2400–2430 Å. and 3075–3100 Å., whereas above 6, a third band is present at 2215–2240 Å., probably due to a shift to longer wave lengths of an acid band existing below 2100 Å. No attempt was made to follow the transition between *p*H's 5.5 and 6. The spectra in distilled water and in 20% alcohol were practically identical with the *p*H 5.5 curve; but in alcohol-petroleum ether solution, three band maxima were found at 2475, 2850, and 3075 Å. The intensities of the several absorption bands are summarized in Table II which lists their wave-length locations in Angström units (Å.) and extinction coefficients ($E^{1\%}_{1\ cm.}$).

this method permits a direct analysis without the necessity of performing customary chemical separations of the compound sought. For example, if the absorption spectrum of a substance is known, and a characteristic intense band chosen, its presence and concentration in a given mixture can be determined merely by measuring the optical density, at this wave length, of an appropriately diluted solution of the preparation against a suitable reference liquid. If the other ingredients of the mixture do not themselves absorb in this region, the reference solution may be the liquid solvent itself. On the other hand, if other components do absorb appreciably, the reference liquid must be constituted identically with the diluted sample, with the exception of the substance being determined. This must be done in order to correct for the light absorption and scattering (turbidity) by the other ingredients of a preparation. In only one type of preparation studied was the direct assay vitiated by the extreme absorption of an inert component; and extraction of this material (an essential oil) with ether was necessary before the assay could be completed.

The absorption band most suitable for the determination of pyranisamine maleate in aqueous medium is located at ≃2435 Å. It is quite intense and, except for a slight shift to shorter wave lengths

TABLE II.—ABSORPTION BANDS OF PYRANISAMINE MALEATE IN SEVERAL MEDIA

Medium	Max. Å.	$E^{1\%}_{1\ cm.}$	Max. Å.	$E^{1\%}_{1\ cm.}$	Max. Å.	$E^{1\%}_{1\ cm.}$
*p*H 4.0	...	...	2400	427	3100	156
*p*H 4.4	...	...	2415	425	3080	145
*p*H 5.0	...	...	2430	423	3070	130
*p*H 5.5	...	...	2430	425	3075	128
*p*H 6.0	2215	465	2435	415	3065	126
*p*H 6.5	2230	424	2440	419	3065	121
*p*H 7	2240	448	2435	436	3055	129
*p*H 11	2230	426	2485	450	3140	124
Distilled water (unbuffered; *p*H ≃ 5.5)	...	...	2435	419	3065	125
20% Alcohol	...	...	2435	423	3075	131
Petroleum ether–absolute alcohol[a]	2475	477	2850	86	3075	122

[a] The spectrum below 2250 Å. was not explored due to the opacity of the solvent employed.

Typical complete spectra are plotted in Figs. 1 (*p*H's 4 and 5.5), 2 (*p*H's 6 and 11), and 3 (20% alcohol and alcohol-petroleum ether), which show optical density vs. wave length. The first two figures also show the absorption spectra of maleic acid in equimolecular concentrations. The spectrum of the free acid is rather insensitive to changes in acidity. It consists of simple end absorption, which decreases gradually as the *p*H increases up to ≃7, beyond which it remains essentially constant. It is clear that the discrete spectrum of the salt, and changes therein, reflect the properties of the pyranisamine itself rather than those of the maleic acid.

APPLICATIONS

Principle.—The absorption spectrophotometric evaluation of pharmaceutical preparations for pyranisamine maleate content, which will be described below, is a typical application of ultraviolet spectrophotometry to the analysis of chemotherapeutic (1) and other types (2) of compounds. In many systems, at lower *p*H's, the extinction coefficient remains quite constant in the *p*H range 4–6.5. Furthermore, the same spectra are found in 20% alcohol and in distilled water (*p*H ≃5.5), indicating an insensitivity of the spectrum to the presence of alcohol and salts from the buffer mixtures. Accordingly, for all work in water or 20% alcohol, an over-all average $E^{1\%}_{1cm.}$ of 422 was employed in calculating concentrations of pyranisamine maleate. For solutions in 1:1 absolute alcohol–petroleum ether, the 2475 Å. band with an $E^{1\%}_{1cm.}$ of 477 was employed. In actual practice, the use of buffers was dispensed with since solutions in distilled water automatically fell in the *p*H range 5–6 The *p*H of aqueous solutions was always checked, however, to assure a *p*H <6.5.

Preparations.—The spectrophotometric assay method for pyranisamine maleate was tested with the pharmaceutical preparations described below. Materials used in compounding these samples were all U. S. P. grade or equivalent.

Tablets.—Two strengths of coated tablets were prepared which contained 25 and 50 mg. of the male-

ate per tablet, respectively, in addition to the customary ingredients such as sugar, starch, certain salts, and a trace of dye. Placebo tablets of identical composition, except for the omission of pyranisamine maleate, were also prepared for reference purposes.

Elixirs.—Several 0.5% elixirs were compounded with alcohol, essential oils, and aromatic elixir. Blank elixirs of identical composition, except for the pyranisamine salt, were always prepared to correct for the incomplete transparency of other components. It was found necessary to use the same lots of oil and aromatic elixir for the blank as were used in formulating the pyranisamine elixir itself because of the varying absorption spectral patterns of different lots.

Anhydrous Petrolatum Ointments.—Several ointments containing 5% of pyranisamine thaleate in white petrolatum were prepared to test the applicability of the spectrophotometric assay method to this type of formulation. No blank ointment was required since the pyranisamine maleate was extracted from the ointment base.

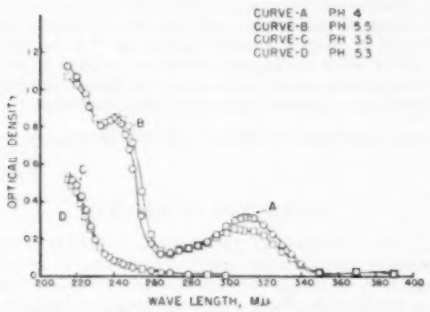

Fig. 1.—Absorption spectra of Pyranisamine Maleate (curves *A* and *B;* 2 mg./100 ml.) and Maleic Anhydride (curves *C* and *D;* 0.488 mg./100 ml.).

Anhydrous Water-Soluble Ointments.—Several 5% ointments of this type were prepared. The base consisted of Carbowax and propylene glycol, and is essentially the same as the Ointment No. 11 base described by Macek, *et al.* (3).

Aqueous Emulsion-Type Ointments.—Five per cent emulsion-type ointments were formulated with wax, higher alcohols, emulsifying agent, glycerin, and water, essentially as specified by the above-mentioned authors (3) for their Ointment No. 2.

Parenteral Solutions.—Sterile aqueous solutions containing 25 mg. of the maleate per milliliter, certain of which contained 0.25% phenol, were also assayed.

PROCEDURES

Tablets.—A solution containing 1 mg. of the maleate per ml. was prepared by allowing 10 tablets to disintegrate in approximately 100 ml. of distilled water and then adjusting the volume to 250 ml. for the 25-mg. tablets and to 500 ml. for the 50-mg. tablets. After thorough mixing, a 10-ml. aliquot was diluted to 500 ml. with distilled water to yield a solution containing ≃2 mg. of pyranisamine maleate per 100 ml., which was then assayed. Optical densities (*d*) of the diluted pyranisamine solutions were measured at 2435 Å. in the Beckmann spectrophotometer using 1-cm. cells, against a reference solution prepared by similar dilution of placebo tablets. The pyranisamine maleate content of the tablets was then calculated from the expression

$$\text{mg. pyranisamine maleate per tablet} = \frac{F \times d}{0.422}$$

where the factor F is 12.5 and 25.0, respectively, for 25- and 50-mg. tablets. The prescribed dilutions lead to a density (*d*) of ≃0.84. Lower densities are

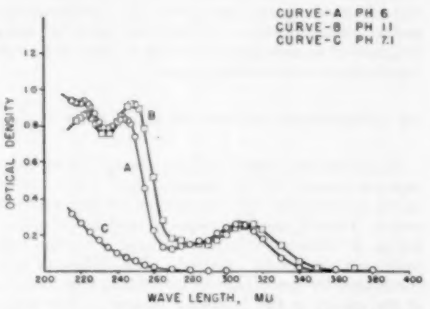

Fig. 2.—Absorption spectra of Pyranisamine Maleate (curves *A* and *B;* 2 mg./100 ml.) and Maleic Anhydride (curve *C;* 0.488 mg./100 ml.).

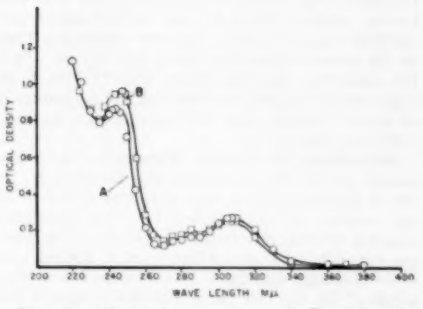

Fig. 3.—Absorption spectra of Pyranisamine Maleate in 20% alcohol (curve *A;* 2 mg./100 ml.) and in 1/1 Petroleum Ether-Absolute Alcohol (curve *B;* 2 mg./100 ml.).

obtained at greater dilutions, which may be chosen to suit the convenience of the operator. For greater dilution than prescribed above, the factor F must be appropriately increased. The same applies to the succeeding preparations.

The placebo tablets were fabricated in order to correct for light absorption or scattering by inert ingredients present. Actually it was observed that,

at the dilutions employed, these effects were inconsequential so that water served as a satisfactory reference liquid. This simplification cannot be taken for granted, however, in all cases; and each new composition must be examined for transparency before placebo tablets are discarded. Another precaution which must always be observed is the maintenance of a final solution pH of < 6.5.

Even when interference by turbidity occurs, placebo tablets can frequently be dispensed with simply by filtering the diluted unknown solution through a sintered glass filter prior to measurement of optical density. Filtration through paper, however, is not permissible because of absorption by the filter paper.

Elixirs.—The 0.5% elixirs were diluted 250 times with 20% alcohol or distilled water to a theoretical content of 2 mg. of maleate per 100 ml. of solution. The blank elixir was identically diluted to produce the reference solution needed for the spectrophotometric measurements. Optical densities (d) were determined as described above for tablets, and elixir concentrations calculated from

$$\text{mg. pyranisamine maleate per ml. of elixir} = \frac{2.5d}{0.422}$$

In certain instances the light absorption by components (especially the essential oils) was so great as to overshadow the absorption by the maleate proper. I nsuch cases, the required precision of preparation of reference solutions became a burden and could not always be attained. This difficulty was eliminated by removing the interfering components of the elixirs in the following manner. Ten milliliters of elixir was diluted with distilled water in a separatory funnel to 100 ml. and shaken with three successive 10-ml. portions of reagent ethyl ether, the organic solvent being removed after each extraction. The remaining aqueous phase was then further diluted (total dilution of 250 times) with distilled water to yield a solution containing 2 mg. of the antihistamine compound per 100 ml. Once the absorbing materials have been extracted, the elixir blank may be discarded and the measurement of optical density may be made against water as reference liquid.

Anhydrous Petrolatum Ointments.—A 5-Gm. sample of the 5% ointments was dissolved in 100 ml. of petroleum ether, and extracted with five 25-ml. portions of distilled water. These combined aqueous extracts containing the pyranisamine maleate were diluted with distilled water to 500 ml. and, after thorough mixing, a 10-ml. aliquot was then adjusted to 250 ml. with distilled water. This solution, containing $\simeq$2 mg. of the antihistamine compound per 100 ml., was then utilized for optical density (d) measurements against distilled water as reference liquid, as described above. The maleate content can then be calculated from the expression

$$\text{mg. pyranisamine maleate per Gm. of ointment} = \frac{25d}{0.422}$$

Anhydrous Water-Soluble Ointments.—A 5-Gm. sample of the 5% ointment was dissolved in distilled water to make 500 ml. of solution, after which a 10-ml. aliquot was further diluted to 250 ml., yielding a solution of $\simeq$2 mg. pyranisamine salt per 100 ml. The reference solution was prepared by solution of 4.75 Gm. of ointment base and dilution as described for the ointment itself. From the density (d) at 2430–2435 Å. of the ointment solution against the blank, the maleate content was calculated as follows:

$$\text{mg. pyranisamine maleate per Gm of ointment} = \frac{25d}{0.422}$$

Aqueous Emulsion-Type Ointments.—A 5-Gm. sample was dissolved and diluted precisely as described for the preceding type of ointment, except that the solvent in this case was a mixture of equal volumes of absolute ethyl alcohol and petroleum ether. The reference solution was obtained by similar solution and dilution of 4.75 Gm. of the blank ointment base. In this case, however, the optical density (d) was measured at 2475 Å., and the concentration of maleate calculated from

$$\text{mg. pyranisamine maleate per Gm. of ointment} = \frac{25d}{0.477}$$

Parenteral Solutions.[1]—The solutions containing 25 mg. of maleate per milliliter were diluted 1250 times to yield a solution containing $\simeq$2 mg./100 ml. of which the optical density (d) at 2435 Å. was determined against water since the phenol did not interfere. Concentration was calculated from

$$\text{mg. pyranisamine maleate per ml. of solution} = \frac{12.5d}{0.422}$$

DISCUSSION OF RESULTS

The usefulness of the method is indicated by typical assay results compiled in Table III which gives in succession the type, number, and history of a preparation, the medium in which the light absorption was measured, the theoretical maleate content, the amount as determined spectrophotometrically, and finally, the per cent deviation from theory. Scrutiny of the last column reveals that only one assay result differs from the expected composition by more than 10%. In most cases, deviations are 5% or less.

The heat and storage stability[2] of certain of these preparations is indicated by the data in Table III. The parenteral solutions, for example, appear to withstand autoclaving for twenty minutes at 120°, as well as subsequent storage for six months at room temperature and 40°, without suffering a loss of pyranisamine content as judged by this method of assay. Similar experiments with elixirs reveal a constant potency even after a year of storage at 40°. Thus the spectrophotometric assay method served to determine both the potency and the stability of these preparations.

SUMMARY

1. A spectrophotometric method of assaying for pyranisamine maleate in pharmaceutical

[1] Prepared by Mr. T. J. Macek of this Laboratory.

[2] The conclusion as to stability is based on the invariance of the ultraviolet absorption spectrum. It is reasonable to assume that, for this type of molecule, any significant degree of decomposition would be attended by measurable changes in absorption spectrum.

TABLE III.—ASSAY RESULTS

Preparation		Storage			Pyranisamine Maleate Content		
Type	No.	Time	Temp.	Medium	Potency	Assay	% Deviation
Elixir	1	Fresh		20% Alcohol	5 mg./ml.	4.90 mg./ml.	− 2.0
	1	Fresh		20% Alcohol	5 mg./ml.	5.29 mg./ml.	+ 5.8
	1	2 mo.	Room	20% Alcohol	5 mg./ml.	5.12 mg./ml.	+ 2.4
	1	2 mo.	40°	20% Alcohol	5 mg./ml.	5.00 mg./ml.	0.0
	1	3 mo.	Room	20% Alcohol	5 mg./ml.	5.18 mg./ml.	+ 3.6
	1	3 mo.	40°	20% Alcohol	5 mg./ml.	5.12 mg./ml.	+ 2.4
	1	6 mo.	Room	Distilled water	5 mg./ml.	5.05 mg./ml.	+ 1.0
	1	6 mo.	40°	Distilled water	5 mg./ml.	4.85 mg./ml.	− 3.0
	1	12 mo.	Room	Distilled water	5 mg./ml.	5.10 mg./ml.	+ 2.0
	2	Fresh		20% Alcohol	5 mg./ml.	4.87 mg./ml.	− 2.6
	3	Fresh		Distilled water	5 mg./ml.	5.00 mg./ml.	0.0
	4[a]	Fresh		Distilled water	5 mg./ml.	5.05 mg./ml.	+ 1.0
	5[a]	Fresh		Distilled water	5 mg./ml.	5.00 mg./ml.	0.0
	6[a]	Fresh		Distilled water	5 mg./ml.	5.00 mg./ml.	0.0
Tablet	1	...	...	Distilled water	25 mg./tab.	23.5 mg./tab.	− 6.0
	2	...	...	Distilled water	25 mg./tab.	23.0 mg./tab.	− 8.0
	3	...	...	Distilled water	50 mg./tab.	48.5 mg./tab.	− 3.0
	4	...	...	Distilled water	50 mg./tab.	47.5 mg./tab.	− 5.0
	5	...	...	Distilled water	50 mg./tab.	46.5 mg./tab.	− 7.0
	6	...	...	Distilled water	50 mg./tab.	52.0 mg./tab.	+ 4.0
	7	...	...	Distilled water	50 mg./tab.	52.5 mg./tab.	+ 5.0
	8	...	...	Distilled water	50 mg./tab.	49.0 mg./tab.	− 2.0
	9	...	...	Distilled water	50 mg./tab.	47.0 mg./tab.	− 6.0
	10	...	...	Distilled water	50 mg./tab.	47.5 mg./tab.	− 5.0
	11	...	...	Distilled water	50 mg./tab.	48.5 mg./tab.	− 3.0
	12	...	...	Distilled water	50 mg./tab.	51.5 mg./tab.	+ 3.0
Petrolatum ointment	1	...	...	Distilled water	50 mg./Gm.	54.5 mg./Gm.	+ 9.0
	2	...	...	Distilled water	50 mg./Gm.	46.5 mg./Gm.	− 7.0
	3	...	...	Distilled water	50 mg./Gm.	51.0 mg./Gm.	+ 2.0
Water-soluble ointment	1	...	...	Distilled water	50 mg./Gm.	51.0 mg./Gm.	+ 2.0
	2	...	...	Distilled water	50 mg./Gm.	48.5 mg./Gm.	− 3.0
	3	...	...	Distilled water	50 mg./Gm.	50.5 mg./Gm.	+ 1.0
Emulsion-type ointment	1	...	...	1:1 Absolute alcohol–petroleum ether	50 mg./Gm.	47.0 mg./Gm.	− 6.0
	2	...	...	1:1 Absolute alcohol–petroleum ether	50 mg./Gm.	56.0 mg./Gm.	+12.0
Parenteral solution	1[b]	Fresh		Distilled water	25 mg./ml.	24.9 mg./ml.	− 0.4
	2[c]	Fresh		Distilled water	25 mg./ml.	25.3 mg./ml.	+ 1.2
	3[b]	Fresh		Distilled water	25 mg./ml.	24.3 mg./ml.	− 2.8
	3[b]	6 mo.	Room	Distilled water	25 mg./ml.	25.1 mg./ml.	+ 0.4
	3[b]	6 mo.	40°	Distilled water	25 mg./ml.	25.0 mg./ml.	0.0
	4[c]	Fresh		Distilled water	25 mg./ml.	24.7 mg./ml.	− 1.2
	4[c]	6 mo.	Room	Distilled water	25 mg./ml.	25.0 mg./ml.	0.0
	4[c]	6 mo.	40°	Distilled water	25 mg./ml.	24.2 mg./ml.	− 3.2

[a] Diluted elixir extracted with ethyl ether prior to assay.
[b] Autoclaved 20 min. at 120°.
[c] Contained 0.25% phenol.

preparations is described. This method is based on the ultraviolet absorption spectrum of the compound.

2. The method has been applied to elixirs, tablets, parenteral solutions, and several types of ointments. In a number of cases, assay results deviate from the theoretical content by less than 5 per cent.

3. The stability of certain preparations containing pyranisamine maleate has been established spectrophotometrically.

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Studies on Pharmaceutical Powders and the State of Subdivision. III. An Evaluation of Pharmaceutical Dispensing Techniques Employed to Increase the Specific Surface Areas of Powders*, †

By J. V. SWINTOSKY‡, S. RIEGELMAN,§ T. HIGUCHI, and L. W. BUSSE

The low-temperature nitrogen adsorption technique was applied to specific surface area determinations on pharmaceutical powders in experiments attempting to evaluate certain comminutative methods used in dispensing practice. Separate samples of zinc oxide, bismuth subnitrate, and sulfanilamide were levigated according to standard techniques with a mortar and pestle, a muller, and a spatula. Their specific surface areas were then determined. Data are presented which indicate the relative efficacy of these comminutative methods in reducing the surface average particle size of these several powders.

No QUANTITATIVE studies of the relative efficacies of the common comminutative procedures employed by dispensing pharmacists appear to have been made in the past. Because of the lack of sensitivity to minor changes in particle size of the previously available methods of analysis,[1] such investigations were necessarily restricted mainly to qualitative observation. A study of this nature, employing the relatively precise nitrogen adsorption technique, is presented.

EXPERIMENTAL

Powders Studied.—The powders chosen for this analysis were U. S. P.[2] Zinc Oxide, U. S. P. Bismuth Subnitrate, and U. S. P. Sulfanilamide. They represent lots of powders with surface average particle sizes of approximately 0.3 μ, 0.6 μ, and 14 μ respectively, as shown by measurements with a low-temperature nitrogen adsorption apparatus (1, 2).

Equipment.—The dispensing equipment used for these studies was new and of conventional type. A 6-inch porcelain mortar was employed. The ground glass muller measured 3 inches across the base, and a 6-inch stainless steel spatula was used. The pill tile used in conjunction with the muller and spatula measured 12 inches square, and all operations of levigation were performed on the ground glass half of the pill tile surface.

Procedure.—Levigation was performed both on the dry materials and on the water pastes of these materials. When water was used, it was added in increments to maintain a desirable consistency of the paste, as one might do in a dispensing operation.

Certain arbitrary procedures were adopted as a means of comparison of these dispensing techniques. One revolution of the pestle, one revolution of the muller, and one sweep with the spatula were all tacitly assumed to require equal physical effort. A single revolution of the pestle consisted of one complete circular movement in the mortar. A single revolution with the muller consisted of one complete circular movement over the ground glass surface of the pill tile. A single sweep with the spatula consisted of a forward motion across the ground glass surface of the pill tile culminated by a backward motion bringing the spatula back to its original starting position. Table I outlines the conditions employed in evaluating the effects of these several dispensing techniques.

After levigation, all powders were dried in a desiccator over anhydrous magnesium perchlorate for a period of at least three weeks. The masses were then crushed to a granular consistency[3] and the specific surface areas were measured with a nitrogen adsorption apparatus as described in previous papers (1, 2) of this series.

RESULTS AND DISCUSSIONS

The results of the measurements on the three pharmaceutical powders after various pretreatment are illustrated in Table II and Figs. 1–3. The effects of the several comminutative methods are seen to differ for the different powders. Zinc oxide did not show an increase in surface area by any of the techniques employed. The specific surface values obtained after pretreatment agreed with the value for the untreated powder. However, bismuth subnitrate showed a marked relative increase in specific surface area with all of the comminutative methods used. Sulfanilamide also appeared to be easily reduced in particle size by all of these methods.[4]

Results on analysis of these few powders would appear to indicate that the extent of specific surface

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† This paper is based in part on a thesis submitted by Joseph V. Swintosky to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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[1] Such as, for example, sieving, sedimentation analysis, etc.

[2] U. S. P. grade chemicals imply U. S. P. XII or XIII.

[3] A mortar and pestle was used to crush the hard masses into granular fragments. It was not necessary to grind the granules to the same condition as the original powder since the nitrogen gas penetrates the granules and measures the absolute surface area as well when the powder is aggregated as when it exists in the nonaggregated condition.

[4] It was observed in the course of these dispensing operations that the bulk volumes of both the zinc oxide and the bismuth subnitrate were reduced to about three-fourths of their original volumes. This observation was not made with the sulfanilamide.

TABLE I.—DESCRIPTION OF COMMINUTATIVE TECHNIQUES EMPLOYED ON SEVERAL PHARMACEUTICAL POWDERS

Powder	Implement	Gm. of Powder Levigated during One Complete Operation	Levigating Medium	Quantity of Liquid, Cc.	Number of Revolutions or Sweeps	Approximate Levigating Time, Min.
Zinc Oxide[a] Sample No. 2A	Mortar and Pestle	25	None	None	300	6
	Mortar and Pestle	25	Water	10	300	6
	Spatula	25	Water	13	300	6
	Muller	25	Water	13	300	6
Bismuth Subnitrate,[a] Sample No. 4A	Mortar and Pestle	23	None	None	300	6
	Mortar and Pestle	23	Water	7	300	6
	Spatula	23	Water	9	300	6
	Muller	23	Water	9	300	6
Sulfanilamide,[a] Sample No. 6A	Mortar and Pestle	10	None	None	300	6
	Mortar and Pestle	10	Water[b]	8	300	6
	Spatula	10	Water[b]	14	300	6
	Muller	10	Water[b]	12	300	6

[a] Analyses of these untreated samples have been reported in Papers I and II of this series.
[b] In levigation of the sulfanilamide pastes, the water used was previously saturated with sulfanilamide.

TABLE II.—SURFACE AREA MEASUREMENTS OF PHARMACEUTICAL POWDERS BY NITROGEN ADSORPTION AFTER TREATMENT BY SEVERAL DISPENSING TECHNIQUES

Material	Method of Treatment	Weight of Sample, Gm.	Sample Number	Analysis Number	V_m, Cc.	Area, M^2/Gm.	Average Area, M^2/Gm.	Density	Surface Average Diameter of Particle, Microns
Zinc Oxide (U. S. P.)	Untreated		2A (see Table I)				3.56	5.47	0.308
	Dry mortar						3.53	5.47	0.311
		21.26		1	0.806	3.51			
		21.26		2	0.814	3.54			
	Wet mortar						3.51	5.47	0.313
		21.32		1	0.806	3.51			
		21.32		2	0.806	3.51			
	Muller						3.52	5.47	0.312
		21.95		1	0.810	3.52			
		21.95		2	0.810	3.52			
	Spatula						3.51	5.47	0.313
		21.06		1	0.806	3.51			
		21.06		2	0.806	3.51			
Bismuth Subnitrate (U. S. P.)	Untreated		4A (see Table I)				0.63	4.93	1.93
	Dry mortar						1.57	4.93	0.775
		19.72		1	0.363	1.58			
		19.72		2	0.362	1.57			
	Wet mortar						0.89	4.93	1.37
		21.27		1	0.204	0.887			
		21.27		2	0.206	0.896			
	Muller						0.82	4.95	1.48
		20.61		1	0.188	0.820			
		20.61		2	0.188	0.820			
	Spatula						0.73	4.93	1.67
		22.28		1	0.169	0.735			
		22.28		2	0.167	0.726			
Sulfanilamide (U. S. P.)	Untreated		6A (see Table I)				0.28	1.49[a]	14.4
	Dry mortar						0.46	1.49	8.75
		31.84		1	0.106	0.461			
		31.84		2	0.105	0.457			
	Wet mortar						0.61	1.49	6.60
		31.70		1	0.142	0.618			
		31.70		2	0.137	0.596			
	Muller						0.45	1.49	8.95
		29.35		1	0.106	0.461			
		29.35		2	0.102	0.444			
	Spatula						0.40	1.49	10.1
		29.34		1	0.093	0.408			
		29.34		2	0.091	0.399			

[a] Density determined with nitrogen adsorption apparatus as described in Paper II of this series.

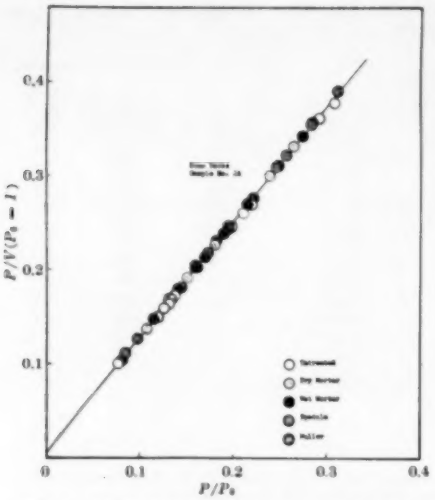

Fig. 1.—BET plots for nitrogen on untreated zinc oxide and on the same zinc oxide treated according to the dispensing techniques indicated. This sample of zinc oxide has previously been reported in Paper I of this series.

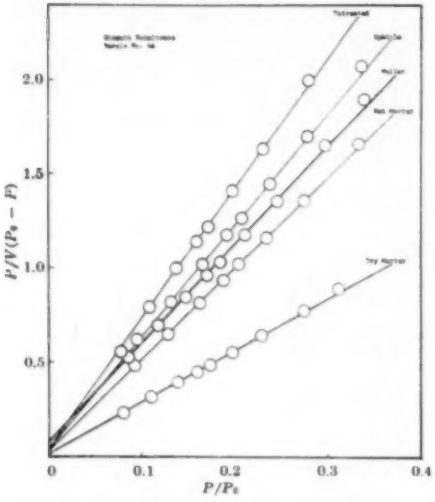

Fig. 2.—BET plots for nitrogen on untreated bismuth subnitrate and on samples of bismuth subnitrate treated according to the dispensing techniques indicated. This sample of bismuth subnitrate has previously been reported in Paper II of this series.

area increase is not influenced by the comminutative technique alone, but probably is also dependent upon certain physical characteristics of the powder such as hardness, brittleness, friability, and initial particle size. The limited data point to the mortar and pestle as the implements of choice where reduction of particle size is desirable. Where only a mixing effect is desired or where aggregates only are to be reduced, the convenient spatula technique would be satisfactory; however, its use for the purpose of increasing the specific surface area of powders during incorporation in a liquid or semisolid base would not seem to be the best procedure.

From this preliminary study it would appear that the mortar and pestle should be used to effect a maximum reduction in particle size prior to incorporation of a powder in another medium such as an ointment base.

SUMMARY

A preliminary investigation is presented on the relative efficacy of the mortar and pestle, the muller, and the spatula as comminutative methods in certain dispensing operations. The powders chosen for this analysis were U. S. P. Zinc Oxide, U. S. P. Bismuth Subnitrate, and U. S. P. Sulfanilamide. The specific surface area measurements were made with a nitrogen adsorption apparatus.

The particle size of zinc oxide was not affected by any of these comminutative methods under the conditions of the experiment. Both bismuth subnitrate and sulfanilamide were reduced in particle size by levigation. The mortar and pestle was most effective and the spatula was least effective in reducing the particle size of the latter two powders.

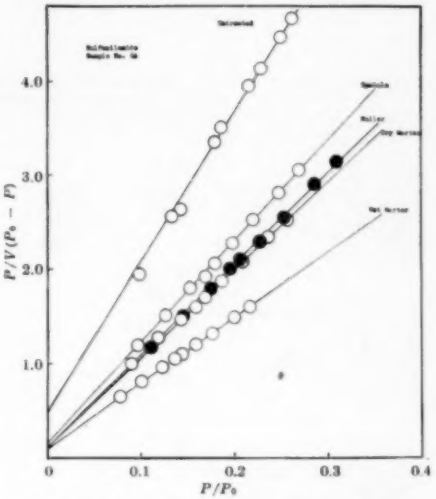

Fig. 3.—BET plots for nitrogen on untreated sulfanilamide and on samples of sulfanilamide treated according to the dispensing techniques indicated. This sample of sulfanilamide has previously been reported in Paper II of this series.

The results would appear to indicate that the extent of particle size reduction is not influenced by the comminutative technique alone, but also by certain physical properties of the powder.

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Microtoxicology. V. Colorimetric Reactions and Optical Crystallographic Properties of Several Antihistaminics of Unrelated Structure*,†

By THOMAS J. HALEY‡ and GEORGE L. KEENAN§

A scheme, based upon colorimetric and precipitation reactions, has been devised for the identification and differentiation of several antihistaminic drugs. The tests employed are described and the results obtained are tabulated.

A RECENT REVIEW (1) showed that although much was known regarding the pharmacological actions of Thephorin (2-methyl-9-phenyl-2,3,4,9-tetrahydro-1-pyridindene), Sc 887 (diethylaminoethyl - 9,10 - dihydroanthracene - 10-carboxylate), 1571F (N′-phenyl-N′-ethyl-N-diethylethylenediamine) and 929F (2-isopropyl-5-methylphenoxyethyldiethylamine), no means was available for their rapid identification. This is also the case with the two new antihistaminics, Diatrin (N′-phenyl-N′-(2-thenylmethyl)-N-dimethylethylenediamine) (2, 3) and Trimeton (1-phenyl - 1 - (2 - pyridyl) - 3 - dimethylaminopropane) (4). However, information is available concerning some of the physical constants and derivatives of all of the above compounds (see Table I).

In view of the scarcity of information on methods for the identification and differentiation of these compounds, it was decided that they should be investigated in the same manner as the other antihistaminics (7–9). Further, so that a comparison of results could be made the methods employed previously were used for this evaluation.

* Received Mar. 17, 1949, from the Medical School, University of California at Los Angeles.
† The authors wish to thank Hoffmann-La Roche, Inc., for the Thephorin, Schering Corporation for the Trimeton, G. D. Searle & Company for the Sc 887, Abbott Laboratories for the 929F and 1571F, and William R. Warner & Company for the Diatrin used in this work.
‡ Medical School, University of California at Los Angeles.
§ Formerly Microanalyst, Food and Drug Administration. Present address: Strongsville, Ohio.

EXPERIMENTAL

The reagents used were prepared as directed by Thienes and Haley (10). To one drop of reagent on a microscope slide or in the well of a spot plate was added about 1 mg. of the compound to be tested and the resulting precipitate or color observed for thirty minutes. Where definite crystalline precipitates were produced, they were allowed to dry and the crystals were then examined by the immersion method for their crystallographic characteristics. The end point with the acidic colorimetric reagents was a charring or the development of a stable color. Where charring occurred only the colors produced prior to this were considered. The results obtained with both types of reagent are given in Table II.

To aid further in the identification of these antihistaminic drugs, photomicrographs of their pure crystals were taken with a Visicam photographic attachment of a Bausch and Lomb binocular microscope using the 16-mm. objective and the 10× eyepiece. See Fig. 1, parts *a*, *b*, *c*, *d*, and *e*, for the photomicrographs.

Inasmuch as the precipitation and colorimetric tests were almost identical for each compound, the optical crystallographic properties of the pure compounds were studied. Trimeton could not be studied in this manner because it was a liquid and it did not form any characteristic crystalline precipitates. Thephorin, Sc 887, and 1571F could not be examined crystallographically due either to the minute size of the particles or to unsatisfactory orientation. This is illustrated in the photomicrographs.

929F HCl

In Ordinary Light.—The substance consists of very small colorless rods and needles.

Characters Shown in Parallel Polarized Light (Crossed Nicols).—The extinction is parallel and the sign of elongation positive.

Characters Shown in Convergent Polarized Light (Crossed Nicols).—Because of the minute size of the crystals, interference figures are rare. Optic sign apparently (+).

TABLE I.—PHYSICAL CONSTANTS OF ANTIHISTAMINIC COMPOUNDS

Compound	Derivative	M. P., ° C.	B. P. ° C.	B. P. Mm.	Reference
Thephorin	Hydrogen tartrate	165–167	....		4
Trimeton	Base	...	135	0.5	3
	Picrate	203–204.5	....		
Sc 887	HCl	170–171	....		5
929F	HCl	121–122	....		...
1571F	HCl	97.5–98.5	....		...
Diatrin	Base	...	183–185	7.0	6
	n_D^{25} 1.5902				
	HCl	186–187	....		

TABLE II.—COLORIMETRIC AND PRECIPITATION REACTIONS OF ANTIHISTAMINICS

Reagent	Trimeton	Thephorin	Sc 887	929F	1571F	Diatrin
Conc. H_2SO_4	Colorless	Canary yellow	Deep orange-yellow	Yellow then pink	Orange	Orange-yellow
Conc. HNO_3	Canary yellow	Colorless	Very faint yellow	Deep blue	Lemon yellow then orange	Brownish red
Mandelin's	Colorless	Greenish blue, then deep blue	Faint greenish yellow	Pale brown with purple center	Bright orange	Orange-red changing to pale yellow
Marquis'	Colorless	Pale brown	Pale brown	Carmine	Pale yellow	Pale yellow
Fröhde's	Colorless	Greenish yellow	Greenish yellow to orange	Pale green then red-violet	Colorless	Orange
Buckingham's	Colorless	Olive green	Green to bluish green	Bluish green then red-brown to black	Colorless	Orange-red
Chloroplatinic acid	Amorphous	Amorphous	Amorphous	Amorphous	Amorphous	Amorphous
Chloroauric acid	Amorphous	Amorphous	Amorphous	Square flat plates of varying sizes	Broad leaflike crystals. Reagent turns blue green	Amorphous
Picric acid saturated aqueous solution	Thin small needles in crosses and bundles	Amorphous	Amorphous	Amorphous	Amorphous	Amorphous

Refractive Indices.—$\alpha = 1.549$, $\beta = 1.556$, $\gamma = 1.570$; all ± 0.002. All the indices are readily found. α usually occurs crosswise, β crosswise, and γ lengthwise.

Diatrin HCl

In Ordinary Light.—The substance consists of colorless, irregular fragments and elongated six-sided rods with obtuse terminations.

Characters Shown in Parallel Polarized Light (Crossed Nicols).—Many of the fragments do not extinguish sharply with crossed nicols.

Characters Shown in Convergent Polarized Light (Crossed Nicols).—Optic axis figures frequently are shown. Optic sign (−).

Refractive Indices.—$\alpha = 1.604$, $\beta = 1.675$, $\gamma = 1.733$; all ± 0.002. Most of the fragments show the α and β indices.

DISCUSSION

Although Trimeton may be differentiated from the other antihistamines by the use of concentrated sulfuric acid, the test cannot be considered specific because such alkaloids as quinine and atropine give a similar reaction with this acid (11). The reactions given by the other compounds investigated with the exception of 929F are very similar to those reported for Benadryl (8), narcotine, solanine, thebaine, and veratrine (11) and it would be difficult to distinguish between the colors observed. However, the reaction between concentrated sulfuric acid and 929F differs in the final color produced from any recorded in the literature so that the acid would aid in differentiating this antihistaminic from the other compounds.

Concentrated nitric acid produces the same color reaction with Trimeton and Sc 887 as it does with narceine, narcotine, physostigmine, strychnine, thebaine, and veratrine so that the test is of little value. This is also the case with 1571F in that codeine, morphine, and papaverine (11) as well as Tagathen (9) produce a yellow then red color with concentrated nitric acid. The test would be of value in distinguishing Diatrin from the other compounds. However, berberine gives a similar red-brown color but the reaction of the two compounds with sulfuric acid would serve to differentiate between them. The color produced by 929F and nitric acid differs from any reported in the literature and would be valuable as a further means of identification.

Thephorin reacts with Mandelin's Reagent similarly to papaverine and quinidine, the final color being identical with that produced by papaverine. However, other tests would differentiate between these two compounds. Mandelin's Reagent produces varying shades of green with berberine, codeine, colchicine, and coniine which are similar to that produced by Sc 887. However, the latter compound shows a stable color while the former compounds have varying final colors. The reaction between 929F and Mandelin's Reagent is sufficiently different from those reported for aconitine and digitalin (11) and Pyribenzamine (8) that it would serve as an additional confirmatory identification test. Both 1571F and Diatrin produce colors with Mandelin's Reagent which are very similar to those seen

Fig. 1 (*a*).—Thephorin.

Fig. 1 (*d*).—929F.

Fig. 1 (*b*).—Sc 887.

Fig. 1 (*e*).—Diatrin.

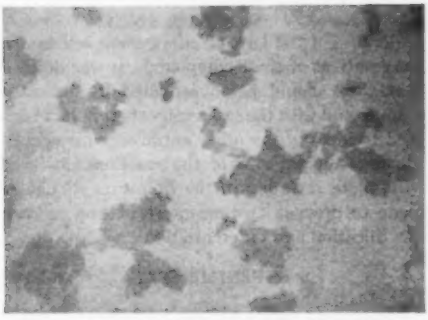

Fig. 1 (*c*).—1571F.

with the halogenated thenyl antihistamines (9) as well as cinchonine, cocaine, pilocarpine, quinine, solanine, thebaine, and veratrine so that the other tests given in Table II would be required before their positive identification was accomplished.

Marquis' Reagent is of value in differentiation of 929F from the other antihistaminic compounds tested (8, 9) and also from many alkaloids (11) because none of the compounds reported give a similar color reaction. However, it would be of but little value in differentiating Thephorin from Sc 887 and 1571F from Diatrin because of the color produced.

Many alkaloids (11) as well as Trimeton and 1571F do not react with Fröhde's Reagent so that this test was of little value. Further, the reactions of Diatrin and Benadryl (8) as well as brucine and solanine (11) are very similar so that differentiation can only be accomplished by observing the final colors produced in all cases. The final colors seen with Thephorin and Sc 887 differentiate these two compounds not only from each other but from colchicine (11). The reaction between 929F and Fröhde's Reagent differentiates it from the other antihistaminics and also from morphine which shows a final color of violet blue (11).

The reactions between Buckingham's Reagent and Thephorin and Sc 887 are similar to that seen with codeine, but other tests of these compounds would serve to differentiate them. The colors observed with both 929F and Diatrin differ from any previously reported (11).

Both chloroplatinic and picric acid are of little value as precipitation reagents with most of the compounds tested. However, Trimeton, which is nonreactive with most of the other reagents employed, gave a crystalline precipitate with picric

acid. The melting point of this picrate would serve as an identifying test for Trimeton.

The crystalline precipitates produced by the reaction between 929F and 1571F and chloroauric acid would serve as additional evidence of their identity because none of the other antihistaminics investigated (7–9) formed crystalline precipitates with this reagent.

SUMMARY

1. A scheme for the identification and differentiation of several antihistaminic drugs has been presented.

2. The colorimetric tests employed enable a rapid identification to be performed.

3. The precipitation reactions are most useful in identifying Trimeton, 929F, and 1571F.

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Microtoxicology. VI. Identification and Differentiation of the Newer Antihistaminic Drugs Related to Benadryl, Pyribenzamine, and Antergan*,†

by THOMAS J. HALEY‡ and GEORGE L. KEENAN§

Seven new antihistaminic drugs related to Benadryl, Pyribenzamine, and Antergan were tested with colorimetric and precipitation reagents and their optical crystallographic properties determined. Because of the similarity of the reactions, their crystallographic properties offer the best means of identification.

SINCE THE advent of Benadryl, Pyribenzamine, and Antergan, many new antihistaminic drugs have been synthesized and investigated both pharmacologically and clinically (1–13). Among these new compounds are Neoantergan (N′-*p*-methoxybenzyl - N′ - 2 - pyridyl - N - dimethylethylenediamine), Neoheteramine (N′-*p*-methoxybenzyl - N′ - 2 - pyrimidyl - N′ - dimethylethylenediamine), Linadryl (β-morpholinoethyl benzhydryl ether HCl), Dec apryn(α-(2-dimethylaminoethoxy)α(methylbenzyl)-pyridine), No. 204 (2-imidazoline-2-methyl benzhydryl ether), Antistine (N′-phenyl-N′-benzylaminomethyl imidazoline), and Dramamine, Sc 1694 (N-dimethylethyl benzhydryl ether-8-chlorotheophyllinate). Of these compounds, only No. 204 has been investigated by the usual toxicological methods (14). However, several of the compounds have their melting points recorded in the literature (see Table I).

Because of the lack of information concerning the reactions of these compounds, it was decided that they should be investigated in the same manner and with the same reagents that had been used to identify the other antihistaminic agents. Further, comparisons of the reactions observed with those recorded in the literature should be made to prevent any misinterpretation in their identification and differentiation.

EXPERIMENTAL

The melting points without references in Table I were determined using a Thiele tube; they are uncorrected.

The reagents used were prepared as directed by Thienes and Haley (17). To one drop of reagent on a microscope slide or in the well of a spot plate was added about 1 mg. of the compound to be tested and the resulting color or precipitate observed for thirty minutes. Where definite crystalline precipitates were produced, they were allowed to dry and the crystals were then examined by the immersion method for their crystallographic characteristics. The end point with the acidic colorimetric reagents was a charring or the development of a stable color.

* Received April 4, from the Medical School, University of California at Los Angeles.
† The authors wish to thank Merck and Co. for the Neoantergan Maleate; Nepera Chemical Co., Inc. for the Neoheteramine HCl; G. D. Searle and Co. for the Dramamine; Wm. S. Merrell and Co. for the Decapryn Succinate; Parke, Davis and Co. for the Linadryl HCl; and Ciba Pharmaceutical Products, Inc. for the Antistine HCl and No. 204 HCl used in this study.
‡ Medical School, University of California at Los Angeles.
§ Formerly Microanalyst, Food and Drug Administration; present address: Strongsville, Ohio.

Where charring occurred, only the colors produced prior to this were considered. The results obtained with both types of reagent are given in Table II.

To aid further in the identification of these antihistaminic drugs, photomicrographs of their pure crystals were taken with a Visicam photographic attachment on a Band L binocular microscope using the 16-mm. objective and the 10 × eyepiece. See Fig. 1, parts *a*, *b*, *c*, *d*, *e*, *f*, and *g*, for the photomicrographs.

Inasmuch as the precipitation and colorimetric tests were very similar for each of the compounds, the optical crystallographic properties of the pure compounds were studied. Due either to the minute size of the particles or to unsatisfactory orientation, optical crystallographic data could not be obtained on Neoantergan Maleate, Dramamine, Decapryn Succinate and No. 204 HCl. This is illustrated by the photomicrographs.

Neoantergan Maleate

In Ordinary Light.—The habit is rod-shaped and irregular fragments.

Dramamine

In Ordinary Light.—The habit is mostly colorless platy material with occasional rods.

Preparation No. 204 Hydrochloride

In Ordinary Light.—The habit is rod-shaped and irregular fragments.

Antistine Hydrochloride

In Ordinary Light.—The habit is rod-shaped and irregular fragments.

Characters Shown in Parallel Polarized Light (Crossed Nicols).—The rods show parallel extinction and the sign of elongations is usually positive. Many fragments do not extinguish sharply with crossed nicols.

TABLE I.—PHYSICAL CONSTANTS OF ANTIHISTAMINICS

Drug	Derivative	M. P., ° C.	B. P., ° C.	B. P., Mm.	Reference
Neoantergan	Base	.....	165–173	0.07	..
	HCl	143–143.5	.....	..	15
	Maleate	100–101	.....	..	..
Neoheteramine	HCl	171–172	.....	..	..
Linadryl A-446	HCl	179–180	.....	..	..
Decapryn	Base	.....	145–153	0.4	..
	HCl	169–170	.....	..	16
	Succinate	98.5–99.5	.....	..	..
No. 204	Base	.....	102–103	..	..
	HCl	203–205	.....	..	14
	Picrate	205	.....	..	..
Antistine	HCl	234 d	.....	..	..
Dramamine Sc 1694	8-Chlorotheophylline	83–85	.....	..	..

TABLE II.—COLORIMETRIC AND PRECIPITATION REACTIONS OF ANTIHISTAMINIC DRUGS

Reagent	Dramamine	Neoheteramine	Neoantergan	Decapryn	Linadryl HCl A 446	Antistine	No. 204
Conc. H_2SO_4	No change	Red	Magenta	Canary-yellow	Canary-yellow with oily orange-red droplets	Colorless	Bright orange
Conc. HNO_3	No change	Colorless	Colorless	Colorless	Colorless	Magenta	Colorless
Mandelin's	Cloudy bluish	Deep pink	Magenta	No change	No change	Brick-red	No change
Marquis'	Cloudy tan	Magenta	Magenta	No change	No change	No change	Pale yellow
Frohde's	No change	Magenta	Magenta	Canary-yellow then orange	Canary-yellow with oily orange-red droplets	Colorless	Canary-yellow with oily orange droplets
Buckingham's	No change	Magenta	Magenta	Canary-yellow then dirty green	Canary-yellow with oily orange-red droplets	Colorless	Canary-yellow with oily orange drops
Chloroplatinic acid	Amorphous	Amorphous	Amorphous then oily globules	Leaf-like agglomerates in bundles	Amorphous	Amorphous	Amorphous
Chloroauric acid	Amorphous	Amorphous	Amorphous	Amorphous	Amorphous	Amorphous	Amorphous
Picric acid sat. aq. soln.	Amorphous	Amorphous	Amorphous	Amorphous	Amorphous	Amorphous	Amorphous

Fig. 1 (*a*).—Neoantergan Maleate.

Fig. 1 (*d*).—Antistine HCl.

Fig. 1 (*b*).—Dramamine.

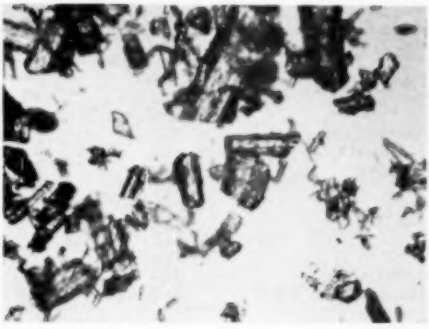


Fig. 1 (*e*).—Linadryl HCl.

Fig. 1 (*c*).—Preparation No. 204.

Fig. 1 (*f*).—Neoheteramine HCl.

Characters Shown in Convergent Polarized Light (Crossed Nicols).—The substance is biaxial and acute bisectrix and optic axis figures are commonly found. 2E is not large. Optic sign (+).

Refractive Indices.—α = 1.683, β = 1.650, γ = >1.688 but <1.733; all ±0.002. α occurs crosswise on rods, β lengthwise, and the value 1.688 crosswise on rods. β is most commonly found.

Linadryl Hydrochloride

In Ordinary Light.—The substance consists of elongated, six-sided, and irregular fragments.

Characters Shown in Parallel Polarized Light (Crossed Nicols).—The fragments invariably extinguish sharply with crossed nicols. Extinction parallel and inclined on elongated forms. The elongation is usually positive.

Characters Shown in Convergent Polarized Light (Crossed Nicols).—The fragments extinguishing sharply with crossed nicols, interference figures are rarely shown. Optic sign apparently (−).

Refractive Indices.—$\alpha = 1.577$, $\beta = 1.631$, $\gamma = 1.672$; all ± 0.002. α common on rods showing parallel extinction (shown crosswise) and β lengthwise on rods showing parallel extinction. α and β are most commonly found.

Neoheteramine Hydrochloride

In Ordinary Light.—The substance is mostly colorless platy material with occasional rods.

Characters Shown in Parallel Polarized Light (Crossed Nicols).—The plates invariably extinguish sharply with crossed nicols. The elongated forms show parallel extinction and positive elongation.

Characters Shown in Convergent Polarized Light (Crossed Nicols).—The sharp extinction with crossed nicols precludes ideal interference figures. Optic sign apparently (−).

Refractive Indices.—$\alpha = 1.612$, $\beta = 1.679$, $\gamma = 1.691$; all ± 0.002. α and β are the most frequent values shown on plates lying horizontally. The γ value occurs much less frequently.

Fig. 1 (g).—Decapryn Succinate.

DISCUSSION

It is difficult to identify Dramamine with any of the reagents used with the exception of Mandelin and Marquis. The colorimetric reactions with these reagents differ from those reported for the alkaloids (18) and the other antihistaminic drugs (19–21). This may have been due to the 8-chlorotheophyllin part of the molecule obscuring or preventing the usual reactions of the Benadryl part (19).

Neoheteramine's reaction with concentrated sulfuric acid is similar to that of Benadryl (19) but the presence of the oily red globules in the latter's reaction makes differentiation easy. The final colors of Histadyl also are sufficiently different so that no mistake can be made in differentiation of the two compounds. Some difficulty might be encountered with curarine, veratrine, solanine, and thebaine (18), but the reactions of these alkaloids with the other reagents used enable an identification of the exact compound to be made. Neoheteramine's reaction with Mandelin's reagent differs from any previous report so that this reaction is the best means for colorimetric identification and differentiation. Marquis' reagent reacts with Neoheteramine to produce the same color shown by Neoantergan and Tagathen (20). With both Fröhde's and Buckingham's reagents, the colors given by Neoheteramine and Neoantergan are the same but they differ from those previously reported. Furthermore, no confusion should result because the other reagents react differently with these two antihistaminics.

Neoantergan reacts with concentrated sulfuric acid similarly to Bromothen, Chlorothen, and Tagathen but the final colors of these latter compounds are considerably different from that produced by Neoantergan. The fact that Neoantergan produced the same color regardless of the reagent used makes its identification very difficult.

Decapryn gives the same reactions with concentrated sulfuric and nitric acids as Thephorin (21) but the two compounds react very differently with the other reagents so that no confusion should result in their differentiation. The reactions of Decapryn, Linadryl, No. 204, and Benadryl (19) with Fröhde's and Buckingham's reagents are very similar, differing only in the final colors produced. However, in the case of Decapryn the greenish color observed with Buckingham's reagent would serve to differentiate this drug from the others. The crystalline precipitate produced when Decapryn was added to chloroplatinic acid is very similar to that seen with Histadyl (20) but the colorimetric reactions of the two compounds are so different that one could not be mistaken for the other.

As pointed out previously, the reactions of Linadryl, No. 204, and Benadryl (19) with Fröhde's and Buckingham's reagents are identical. Furthermore, their reactions with concentrated sulfuric acid are sufficiently alike to make differentiation difficult. The structural similarity of the three compounds may be the reason for the identical colorimetric reaction.

From the structural formulas on Page 388, it would seem that the colorimetric reaction is due to the benzhydryl ether portion of the molecule, because this is the part of the molecule which is the same in all three compounds. Furthermore, when this part of the molecule is changed by the substitution of a pyridyl group for a phenyl group, as in Decapryn, the color produced is different.

The colorimetric reaction of Antistine with concentrated nitric acid differs from those of the other antihistaminics (19–21) and the alkaloids (18), and thus can be used for identification purposes. Antistine's reaction with Mandelin's reagent differs from those reported for the other antihistaminics but is similar to the reactions of atropine, burcine, morphine, narceine, narcotine, picrotoxin, solanine, thebaine, and veratrine. However, observation of the preliminary as well as the final colors will aid in differentiating these alkaloids from Antistine. In addition, use of the other tests will enable the toxicologist to identify the exact compound.

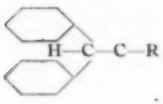
Although Cavallini and Mazzucchi (14) reported precipitation reactions between No. 204 and ammonium molybdate, picric acid, mercuric chloride, chloroauric acid, Mayer's reagent, chloroplatinic acid, and Reinecke's salt, only those precipitation reagents shown in Table II were investigated, because amorphous precipitates are of little value for identification purposes. It should be pointed out

also that Benadryl and Pyribenzamine have previously been shown to form similar amorphous precipitates with most of the reagents listed above (19). However, results in Table II confirm those of Cavallini and Mazzucchi (14).

properties were the most useful means for positive identification.

2. The colorimetric tests reported are useful in classification but should be combined with melting-point determinations for accurate identification.

3. The precipitation reagents used were of little value due to the formation of amorphous precipitates. However, Decapryn Succinate does form a crystalline precipitate with chloroplatinic acid.



Drug	R
Benadryl	$-CH_2-CH_2-N(CH_3)(CH_3)$
Linadryl	$-CH_2-CH_2-N$(morpholine)
No. 204	$-CH_2-C(=N-CH_2)(-NH-CH_2)$

SUMMARY

1. A method for the identification and differentiation of seven new antihistaminic drugs related to Benadryl, Pyribenzamine, and Antergan has been presented. Where the crystals were of sufficient size, the optical crystallographic

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Studies on Indian *Datura**

By R. CHATTERJEE† and J. K. LAHIRI

Certain *Datura* species from Bengal compare favorably in the per cent of alkaloids with the same species found elsewhere.

THE MOST commonly occurring Indian species of *Datura*, which is hitherto known as *D. fastuosa* L., is synonymous with *D. fastuosa* var. *alba* and *D. alba* Nees. *D. fastuosa* of Linnaeus and of other authors is now treated merely as a variety of the type, namely, *D. metel* Linn. var. *fastuosa* (Linn.), (1).

THE ALKALOID CONTENT

It has been found that either hyoscyamine or hyoscine is the main constituent of the species of

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Datura. Table I shows the different species of *Datura* with respect to their main alkaloid. In the tables the old nomenclature has, however, been continued to avoid confusion.

TABLE I.—CLASSIFICATION OF *Datura* SPECIES

Name of the Species	Locality of the Species Examined
A. Species of *Datura* with hyoscyamine as the main alkaloid	
1. *D. stramonium*	America (2), British Columbia (3), Norway (4), Germany (5), Switzerland (5), Egypt (6), South Africa (7), India (8), China (9), Shanghai (10), and New Zealand (11)
2. *D. stramonium* var. *inermis*	Poland (12)
3. *D. fastuosa*	Holland (13)
4. *D. metel*	Patiala (India, 8), Germany (24, 25)
5. *D. latula*	South Africa (7) and Peru (19)
6. *D. sanguinea*	Bogota (S. America, 14) and Peru (19)
7. *D. quercifolia*	Germany (24)
8. *D. ferox*	(27)
B. Species of *Datura* with hyoscine as the main alkaloid	
1. *D. arborea* (*Brugmansia suaveolens or B. candida*)	Germany (24, 25), Peru (19), and Bogota (14)
2. *D. meteloides*	Europe (26)
3(*a*). *D. fastuosa* var. *flor. coerul. plen.*	Assam (India, 8), Europe (8), Germany (23)
3(*b*). *D. fastuosa* var. *flor. alba*	Bengal (India) and Philippines (15)
4. *D. alba* Nees	Philippines (15, 18), Formosa (16), and China (22)
5. *D. innoxia* Miller	Ohio (17)
6. *D. metel* (*D.* var. *fastuosa*)	Lyallpur (India, 8), Germany (25)

It is interesting to note that even in one or two isolated cases, the nature of the alkaloid changed with respect to place; for instance, seeds of *D. metel* from Patiala (8) yielded hyoscyamine, although they usually produced hyoscine; and again *D. fastuosa* which is known to contain hyoscine had been found to contain hyoscyamine in Holland (13). The case of *D. metel* is particularly noteworthy, since even in places which are not geographically distant from one another, e.g., Lyallpur and Patiala, *D. metel* has synthesized a different alkaloid, obviously from the variation of soil and climate.

Since *D. fastuosa* (which is now called *D. metel* var. *fastuosa*, samples of which were assayed by the present writers) grows wild in Bengal, a tropical part of India, it was thought that a chemical examination of the different parts of the plant might lead to finding an economic source of either hyoscyamine or hyoscine.

With due attention to botanical controversy (1) it has been found that both the white and purple flowering *D. fastuosa* (now called *D. metel* var. *fastuosa*) contain hyoscine as the main alkaloid and that the quantity of the alkaloid is more or less the same in both the varieties. The average total alkaloids in the seeds is 0.34 per cent of which 77 per cent is hyoscine. (See Table II.)

EXPERIMENTAL

The Material.—Both the purple and white flowering plants of *Datura metel* var. *fastuosa* were collected from different places in the suburbs of Calcutta and from other parts of Bengal.

Preparation of Material for Estimation of Total Alkaloids.—Different parts of the plants, roots, leaves, fruits, and seeds were air dried, finely powdered (40 mesh), and kept in a vacuum desiccator over anhydrous calcium chloride before subjecting each to alkaloidal assays.

Estimation of Alkaloids.—Thirty-gram portions of powdered and dried drug were macerated in a strong-walled stoppered bottle with 300 cc. of ether, and after shaking vigorously, 15 cc. of 10% ammonia was added. The mixture was frequently shaken for two hours. The ethereal solution was decanted off and after a short drying with anhydrous sodium sulphate, it was filtered; 200 cc. of the filtered ethereal solution (i.e., 20 Gm. of drug) was evaporated to a few cc. on a water bath, 10 cc. of 0.5% hydrochloric acid was added, and the mixture was warmed again until the smell of ether disappeared. The acid solution was filtered into a separating funnel; the flask and the filter were washed thrice, each time with 5 cc. of water; the solution was then made slightly alkaline by carefully adding a freshly prepared saturated solution of sodium bicarbonate. The solution was extracted thrice, each time with 20 cc. of ether. The aqueous solution (*A*) containing hyoscyamine was put aside.

The ethereal solution was evaporated on the water bath to a few cc. Ten cubic centimeters of 0.5% hydrochloric acid was added and the mixture was warmed until the smell of ether disappeared. The acid solution was taken in a separating funnel, made alkaline with saturated solution of sodium carbonate as before, and extracted thrice with ether, each time with 20 cc. The aqueous solution still contained small quantities of hyoscyamine and was added to solution (*A*). The ethereal solution was treated as before, for a third time, and then dried with anhydrous sodium sulfate, evaporated on a water bath. The residue, which is hyoscine, was dissolved in 2 cc. of absolute alcohol and diluted with 10 cc. of water. The hyoscine was estimated by titration with decinormal hydrochloric acid with methyl red-methylene blue as indicator (1 cc. of 0.1 *N* HCl = 0.0303 Gm. hyoscine).

The combined aqueous solution (*A*) was treated with 3 cc. of concentrated ammonia and shaken thrice with chloroform, each time with 30 cc. The chloroform solution was shaken with 0.5 Gm. gum tragacanth, and after standing for about thirty minutes, it was filtered into a flask. The chloroform solution was evaporated completely on the water bath and the last traces of chloroform were removed by blowing air. The residue, hyoscyamine, was kept in a desiccator for several hours and then dissolved in 20 cc. of 90% alcohol. Ten cubic centi-

TABLE II.—THE PER CENT OF ALKALOIDS OF *D. fastuosa* (*D. metel* L. VAR. *fastuosa*) FROM DIFFERENT SOURCES

Source	Parts Used	Total Alkaloid, %	Hyoscine, %	Hyoscyamine, %	Reference, %
1. Europe	Seeds	0.200	0.100	0.100	(8)
2. Germany					
(*a*) Purple flowering	Seeds	0.254	0.220	0.034	(23)
(*b*) White flowering	Seeds	0.223	0.200	0.023	(23)
3. Holland	Seeds	0.149	...	0.149	(13)
4. Philippines	Roots	0.248	...	...	(15)
	Leaves	0.221	...	...	
	Fruits	0.224	...	...	
	Seeds	0.443	...	...	
White flowering	Roots and wood	0.170	...	...	(20)
	Leaves	0.210	...	...	
	Seeds	0.465	...	...	
D. alba Nees	Leaves	0.210	...	...	(21)
	Fruits	0.175	...	...	
	Seeds	0.393	...	...	
5. China					
D. alba Nees	Flowers	0.485	...	...	(22)
6. Formosa	Seeds	0.260	0.240	0.020	(16)
7. Patiala (India)	Fruits	0.120	0.120	...	(8)
	Seeds	0.250	0.080	0.170	
8. Lyallpur (India)	Leaves	0.250	0.250	Traces	(8)
	Seeds	0.230	0.230	Traces	
9. Assam (India)	Roots	0.101	...	...	(8)
Purple flowering	Leaves	0.119	...	...	
	Fruits	0.202	...	...	
10. Bengal (India)					
(*a*) Purple flowering	Roots	0.276	0.180	0.096	
	Leaves	0.143	0.091	0.052	
	Fruits	0.252	0.204	0.048	
	Seeds	0.383	0.288	0.095	
(*b*) White flowering	Roots	0.240	0.151	0.089	
	Leaves	0.105	0.079	0.026	
	Fruits	0.223	0.192	0.031	
	Seeds	0.300	0.248	0.052	

meters of this solution were taken into a flask and diluted with 20 cc. of water. The alkaloid was estimated by titration with decinormal hydrochloric acid with methyl red-methylene blue as indicator (1 cc. of 0.1 *N* HCl = 0.02892 Gm. hyoscyamine).

The identity of both hyoscine and hyoscyamine was established by adding aqueous picric acid to the respective alkaloid solution after neutralization to obtain the picrates; then the crystals were purified by crystallizing twice from water. The picrate of hyoscine obtained had m. p. 186°, and that of hyoscyamine obtained had m. p. 163°. Pure hyoscine picrate has m. p. 188°, and hyoscyamine picrate has m. p. 165°.

SUMMARY

1. Both the purple and white flowering *D. metel* L. var. *fastuosa* (L.) contain approximately the same per cent of hyoscine and hyoscyamine in trace amounts.

2. The average total alkaloid in the seeds is 0.34 per cent, of which 77 per cent is hyoscine.

3. *Datura* species from Bengal compare favorably in the per cent of alkaloids with the same species found in other parts of India, e.g., Assam, Lyallpur, Patiala, and in different places—Japan, Philippines, Germany, and Holland.

4. It is recommended that the seeds of *D. metel* var. *fastuosa* may be used as a source of hyoscine.

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Determination of Theobromine in Theobromine and Sodium Salicylate*

By C. W. BELL†

A volumetric, acidimetric method is proposed as a substitute for the present N. F. gravimetric method. The proposed method features several advantages such as, ease of operation, saving of time, elimination of an empiric correction factor, and a high degree of precision and accuracy. Results are submitted to show that the present N. F. gravimetric method gives values which are erratic and usually high. A review of the literature on methods for the determination of theobromine is also included.

THE DETERMINATION of theobromine in theobromine sodium salicylate has been a question of controversy since the compound was first introduced to the medical profession in 1890 under the trade name "Diuretin." A wide variety of methods were proposed from time to time, were used for a while, only to be discarded for newer methods. A survey of the literature on the subject reveals that most of the earlier methods were of a gravimetric nature which simply involved dissolving the soluble salt in a small amount of water, precipitating the theobromine by adding acid, washing and weighing the residue without regard for the theobromine which might be dissolved in the wash water. When the compound was brought within the scope of the United States Pharmacopœia in 1916 (1) an improved gravimetric method was adopted as the official method. This method provided for the use of an empiric correction factor to compensate for the loss of theobromine. Many other attempts were made to standardize and improve the gravimetric method. In 1920 Schaap (2) suggested precipitation of the theobromine with zinc sulfate instead of one of the mineral acids. In 1921 Bennett and Windle (3) attempted to improve upon the United States Pharmacopœia IX method by more closely standardizing the conditions of the precipitation and the washing of the theobromine. They also proposed the use of a correction factor which would allow for a 14% loss in theobromine. Deér (4), in 1927, suggested the use of boric acid instead of a mineral acid for the precipitation of the theobromine and proposed a different correction factor. In the same year Mikó (5) carried out the precipitation in an ethyl alcohol, or ethyl alcohol-ether medium, and eliminated a correction factor. During this period when most of the investigators were presenting modifications of the gravimetric method, Emery and Spencer (6) announced an entirely new approach to the problem. This consisted of a volumetric method depending upon the formation of theobromine periodide, supposedly having the formula $C_7H_8O_2N_4 \cdot HI \cdot I_4$, by the action of iodine and potassium iodide upon a solution of theobromine in glacial acetic acid, and the determination of the excess of standard iodine with thiosulfate. The results by this method, however, do not show that it gives consistent results in the hands of various operators, and it has the very decided disadvantage that it would return as theobromine any substance which is capable of combining with iodine in acetic acid solution. To overcome these objections, as with the older gravimetric method, many modifications were introduced by a number of workers (7–12). None of these modifications were universally accepted as giving satisfactory and concordant results.

The first real important advancement in the determination of theobromine was the publication in 1930 of Boie's acidimetric method (13). This method is based on the fact that silver nitrate reacts with theobromine to form insoluble silver theobromine with the simultaneous quantitative liberation of nitric acid according to the following reaction: $C_7H_8O_2N_4 + AgNO_3 \rightarrow AgC_7H_7O_2N_4 + HNO_3$. The liberated nitric acid can then be titrated with standard alkali. The method has stood the test of time and has been slowly but en-

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† The author wishes to thank Mr. R. O. Hauck for his constructive comments in carrying out this study.

thusiastically received by a number of observers (14–17). The method now has official status through acceptance by the American Association of Official Agricultural Chemists for the determination of theobromine in theobromine calcium (18). In 1933, Mikó (19) proposed a method basically resembling the method of Boie in that an excess of standard silver nitrate is added to a solution of theobromine and sodium salicylate, the precipitated silver theobromine is filtered and the excess of silver nitrate determined in an aliquot portion of the filtrate with thiocyanate using ferric alum as an indicator. The accuracy of this method was confirmed by Zilberberg and Faĭnshteĭn in 1939 (20).

A review of the methods proposed for the determination of theobromine would not be complete without reference to two methods entirely different from the ones mentioned above: first, the methylation procedure reported by Self and Rankin (21) and later adopted as the official method of the British Pharmacopœia (22); second, a gravimetric method reported by Richardson and Campbell in 1942 (23) which utilizes the classical complex salt formation between theobromine and phosphotungstic acid. acid. When official recognition of Theobromine Sodium Salicylate was transferred from the United States Pharmacopœia to the National Formulary in 1942, the gravimetric assay method was not altered; in fact, the method of the present National Formulary (24) is essentially the same as when the drug first became official in 1916, although the method has long been acknowledged to be unsatisfactory due to the varying amounts of theobromine which are lost in the process. It has been the experience in these laboratories that more than ordinary care must be exercised in following the prescribed procedure in order to obtain consistently reproducible results; even if such results can be obtained there is always the likelihood of a constant error due to the fact that an empiric correction factor has to be used. A critical survey and investigation of practical methods for the determination of theobromine in the compound have led to the conclusion that of all the methods investigated, Boie's acidimetric method gives the fastest and most accurate results. The present study was undertaken in order to compare this method with the present N. F. VIII gravimetric method using the nitrogen content, as determined by Kjeldahl, as a standard of comparison.

EXPERIMENTAL

Ten commercial samples were used in this investigation, six of which were Theobromine and Sodium Salicylate N. F. VIII, made by two different manufacturers, and the other four were samples of "Diuretin." All of the samples were assayed for their theobromine content by the three methods listed below.

Gravimetric Method of N. F. VIII.—The procedure of the N. F. VIII, page 532, was carefully followed, with particular attention to details.

Method Based on Nitrogen Content.—The Kjeldahl-Gunning-Arnold method with a preliminary digestion on a water bath as proposed by the author (25) was used. This method is as follows: Place an accurately weighed sample of about 350 mg. of theobromine sodium salicylate in a 500-cc. Kjeldahl flask and add 15–18 Gm. of anhydrous sodium sulfate together with 1 Gm. of cupric sulfate and 25 cc. of fuming sulfuric acid. Heat the flask with contents on a boiling water bath for thirty minutes, occasionally shaking it so that the digestion mixture comes in direct contact with all of the sample. Remove the flask from the water bath and place in an inclined position in the usual manner. Heat the mixture gently until frothing ceases, making sure that the temperature is maintained below the boiling point of the acid. Gradually increase the heat until the acid boils briskly, and digest for two hours after the mixture has turned green or until oxidation is complete. After cooling, dilute with about 100 cc. of water and add a few pieces of granulated zinc to prevent bumping. Add sufficient concentrated sodium hydroxide solution to make the reaction strongly alkaline, pouring it down the side of the flask so that it does not immediately mix with the acid solution. Connect the flask to a condenser by the use of a connecting bulb and place the tip of the condenser below the surface of 50.00 cc. of 0.1 *N* sulfuric acid contained in the receiver; carefully mix the contents of the flask by shaking and distill until all of the ammonia has passed into the measured excess of standard acid. Titrate the excess 0.1 *N* sulfuric acid with 0.1 *N* sodium hydroxide solution using methyl red T. S. as the indicator. Each cc. of 0.1 *N* sulfuric acid is equivalent to 1.401 mg. of nitrogen or 4.504 mg. of theobromine.

Suggested Acidimetric Method for Next Revision of N. F.—Transfer an accurately weighed sample of about 1 Gm. of theobromine sodium salicylate, previously dried to constant weight at 110°, to a 500-cc. wide-mouth Erlenmeyer flask. Add 75 cc. of distilled water and 40 cc. of approximately 0.1 *N* sulfuric acid. Boil for two to three minutes, washing down the sides of the flask with three 15-cc. portions of distilled water. Cool quickly to about 40° and add 1 cc. of phenol red T. S. Add a slight excess of 0.1 *N* sodium hydroxide to make the solution alkaline and then carefully adjust the *p*H of the solution by adding the minimal amount of 0.1 *N* sulfuric acid required to produce a lemon-yellow color. Add 35 cc. of 0.1 *N* silver nitrate and titrate the liberated nitric acid slowly with 0.1 *N* sodium hydroxide, to the first sign of a bluish red color. Approach the end point drop by drop. Each cc. of 0.1 *N* sodium hydroxide is equivalent to 18.017 mg. of theobromine.

RESULTS AND DISCUSSION

Table I gives the results on the determination of theobromine in the ten commercial samples by the

three methods. The results are typical of those obtained in these laboratories during fifteen years of experience with the methods outlined above. The tabulated results are averages of at least two, and in some cases three, determinations, all of which were made on the anhydrous sample.

A study of Table I shows that the proposed acidimetric method yields results which are in excellent agreement with the standard method using nitrogen as a basis for calculating theobromine. On the other hand, a similar comparison with the present National Formulary method shows that of the ten samples tested the results average 1.39% higher, on an absolute basis, than by the other two methods. This corresponds to a relative difference of approximately 3%. One can readily see from Table I that all of the samples meet the minimum requirement of 46.5% of theobromine when tested by the official method. However, when tested by the proposed acidimetric and nitrogen methods some of the samples fail to meet the official standards while others would barely pass. In other words, some of the theobromine sodium salicylate being used today would fail to meet the official requirements of the National Formulary if a more accurate method for the determination of the theobromine content were adopted. This again emphasizes the inadequacy of the official method.

In addition to being more accurate, the proposed volumetric method presents several other important practical advantages. It is simple in operation, convenient to use and, most of all, very rapid. A complete theobromine determination can be run by the acidimetric method in a matter of minutes whereas several hours elapse before results can be obtained with the official gravimetric method. Another important practical advantage of the method is its wide applicability in determining theobromine in combination with various other medicaments and also in various pharmaceutical forms such as tablets. The usual tablet diluents and excipients do not interfere.

TABLE I.—DETERMINATION OF THEOBROMINE BY THREE METHODS

No.	Sample	Theobromine Content from Nitrogen, %	Proposed Acidimetric Method: Theobromine Content, %	Proposed Acidimetric Method: Difference, %	Present N. F. Method: Theobromine Content, %	Present N. F. Method: Difference, %
1	Theobrom. et Sod. Salicyl. N. F. VIII "A"	46.67	46.69	+0.02	48.92	+2.25
2	Theobrom. et Sod. Salicyl. N. F. VIII "B"	46.28	46.19	−0.09	47.15	+0.87
3	Theobrom. et Sod. Salicyl. N. F. VIII "C"	47.03	47.14	+0.11	48.81	+1.78
4	Theobrom. et Sod. Salicyl. N. F. VIII "D"	46.51	46.39	−0.12	47.52	+1.01
5	Theobrom. et Sod. Salicyl. N. F. VIII "E"	46.85	46.76	−0.09	48.03	+1.18
6	Theobrom. et Sod. Salicyl. N. F. VIII "F"	46.19	46.25	+0.06	47.70	+1.51
7	Diuretin "A"	46.40	46.25	−0.15	48.04	+1.64
8	Diuretin "B"	46.95	47.05	+0.10	48.33	+1.38
9	Diuretin "C"	46.50	46.49	−0.01	47.61	+1.11
10	Diuretin "D"	46.64	46.70	+0.06	47.80	+1.16
Mean difference		...	...	−0.01	...	+1.39

SUMMARY

1. The literature on methods for the determination of theobromine in Theobromine and Sodium Salicylate is reviewed.

2. The disadvantages of the present National Formulary method are discussed.

3. Ten commercial samples were analyzed for their theobromine content by three different methods: (1) the present National Formulary VIII method, (2) the proposed acidimetric method, and (3) from the nitrogen content as determined by a modification of the Kjeldahl method. The results show that the present official National Formulary method gives high results while the acidimetric method gives results which are in close agreement with those calculated from the nitrogen content.

4. Compared with the official method, the proposed procedure features ease of operation, saving of time, and a high degree of precision and accuracy.

5. The author recommends that a study of the proposed method be made with the view of its adoption as a standard National Formulary procedure.

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Scotch Mint and Spearmint. A Comparative Study of Cultural, Morphological, and Histological Characteristics of Species of *Mentha* Growing in Florida. III*

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FOR OVER a century, the United States Pharmacopœia has recognized as Spearmint only the one species, *Mentha spicata* L. (*M. viridis* L.). Within recent years, however, the commercial crude drug and its volatile oil on the one hand and the current Pharmacopœial description on the other have not always corresponded in all respects to the species named. A "variety" known as "Scotch Spearmint" has become popular with the American growers. As this is apparently a true species, quite distinct from *M. spicata* (the reasons are detailed in this paper), it would seem manifest that recognition of and data for this entity should appear in the official monographs.

STUDIES

The species *Mentha spicata* is well characterized taxonomically, i.e., differences of various specimens in the chief botanical characters are relatively slight. Its inflorescence is made up of verticillasters (pseudowhorls), comprising a "spike" which is characteristically compact and continuous except close to the base, but occasionally is more or less broken by growth of the internodes separating the "whorls." Always, however, the spiciform habit in this species is pronounced, with no foliage leaves composing part of the inflorescence proper. The glabrous or subglabrous foliage leaves stand below the inflorescence and are definitely sessile, or at the most (such as on lateral axes) with inconspicuous petioles.

The author's interest in the subject was initially attracted by the U. S. P. XII monograph, where petioles up to 12 mm. in length were permitted. Up to and including U. S. P. VIII, the leaves were described as subsessile or "usually sessile." U. S. P. IX, X, XI, and XIII permit petioles up to 5 mm. long. However, the author's studies (described later) show that *M. spicata* leaves do not ordinarily exhibit petioles longer than 3 mm., leaves with longer stalks originating from a separate species, presumably *M. cardiaca* Gerard, forming the main subject of this paper. This is only one of several significant differences in the species, to be noted later.

Entities Having Spearmint Odor.—Several varieties of *Mentha spicata* L. em. Hudson (*M. spicata* (L.) Hudson) have been grown for their herb and volatile oil. Of the 29 varieties of the species listed by John Briquet (1) alone, the following are said to be cultivated to some extent: var. *tenuis* (Mx.) Briquet, said to be a common U. S. cultigen; var. *trichoura* Briq., claimed common in England; var. *crispata* (Schrad.) Briq. (*M. crispata* Schrad.),[1] grown in Germany; and other varieties grown to a smaller extent in civilized temperate countries.[2]

The penetrating and characteristic aroma and taste of spearmint[3] are possessed in common by a number of species of *Mentha*, some of which are regularly used, others not. At least one plant from a different genus and family also possesses the characteristic odor of spearmint, viz., *Tanacetum balsamita* L. (*Compositae*). Spearmint odor-taste is said to be observed in *M. longifolia* (L. pp.) Hudson

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[1] There has been considerable confusion over the Crisped or Curled Mint of Germany and other Continental countries. While most Folium Menthae Crispae originates from *M. spicata* var. *crispata*, a certain amount also appears to come from *M. longifolia* Huds. var. *undulata* Briq. (2) and from other species, some of which have actually a quite different aroma from that of spearmint. Tschirch (3) has made the rather broad generalization that all crisped mints have a spearmint-like odor. Briquet compiled for him (3) 20 varieties included in 9 species (inclusive of *M. spicata* with 6 such varieties), which have crisped leaves. Flueckiger (4) noted that the German Pharmacopœia II recognizes under *M. crispa* any *Mentha* species with crisped leaves, as long as it has sufficient carvone in its oil. Hence, *M. spicata* varieties with flat uncrisped laminas and *M. Cardiaca* were excluded.

[2] Topitz (5) listed 13 forms of *M. spicata;* others were proposed by Opiz, Lejeune and Courtois, H. Braun, *et al.* In addition, other varieties are occasionally referred to by horticulturists, e.g., "var." *anisatus* ("anise-scented mint"), etc.

[3] Pleasant to most but not all people, and described by some as coarse and unpleasant.

(*M. sylvestris* L.),[4,5] and some varieties, such as *mollissima*;[4] *M. niliaca* Jacq. f. *nemorosa* (Willd.) Briquet (= *M. longifolia* (L.) Hudson var. *nemorosa* ? = *M. nemorosa* Willd. ?);[4] *M. rubra* Hudson (possibly synonymous with *M. Cardiaca*);[4] *M. gentilis* L. (6);[6] *M. aquatica* L. var. *crispa* Benth. (*M. crispa* L.);[6] *M. alopecuroides* Hull (7);[4] *M. verticillata* L. (= *M. thuringiaca* H. Br. & Top. = *M. dalmatica* Tausch f. *thuringiaca* H. Br. & Top.);[6,7] and in *M. cardiaca*.[4] In addition, the following species have sometimes been said to have spearmint properties: *M. javanica* Blume (*M. arvensis* L. var. *javanica* Benth.);[8] a few forms of *M. canadensis* L.;[6] *M. pratensis* Sole;[8] *M. rotundifolia* (L.) Huds.[6] (8); *M. villosa* Huds. (7, 9);[8] and others.[9] As happens not uncommonly, it is likely that the record here is confused by frequent wrong identifications,[10] resulting from careless or hurried work, or the lack of specialized study of adequate reference materials.

The odor of Spearmint (etc.) was frequently stated to resemble that of Basil (*Ocymum* species),[6] hence the use of such synonyms as *M. ocimiodora* Opiz (= *M. spicata* L. per De Candolle (12)).

Spearmint plantings in U. S.—Field studies in Michigan undertaken by the author have confirmed that there are now two types of spearmint grown in that area: (*a*) "Native (American) Spearmint," also known variously as "Common Spearmint," "Ordinary Type," "True Variety" (so called because the original sort used), "American spearmint," etc. This is the well-known *M. spicata*, possibly in more than one variety (Fig. 1). Undoubtedly, this is the first type of spearmint found in America, having apparently been reported in 1672 by John Josselyn and *ca.* 1739 by Gronovius (13) as a naturalized plant, later by others (14, 15). (*b*) Scotch (Scottish?) Spearmint, also sometimes known as "Highland Mint,"[11] etc. A better common name would be Scotch mint, since this would more emphatically indicate its specific distinction from spearmint (Figs. 2, 3).

Fortunately, a brief account of the early history of Scotch mint was obtained from Mr. Richard F. Stroud, of Kalamazoo, Mich. (personal communication, Dec. 10, 1947), who has had an experience of about fifty years in the cultivation of the mints and other medicinal plants. He states:

Fig. 1.—*Mentha spicata* L. Specimen from cultivation near Lafayette, Indiana, 1941.

Fig. 2.—Scotch Spearmint. *Mentha cardiaca* Gerard. Specimen collected in Michigan and lent by A. M. Todd Company, Kalamazoo, Mich., 1944.

[4] Aroma corroborated by the author.

[5] The older English authors sometimes referred to this species as spearmint. For instance, Gerard (10) said: "The leaues (*Sic!*) of Speare Mint are long like those of the Willow tree but whiter, softer, and more hairie."

[6] Could not be confirmed from available specimens. Negative findings are not however reliable, since some specimens of mint lose their aroma after only a few years, while in other cases the odor is manifest after scores of years of storage, sometimes even after a century!

[7] Var. *strabala* Briq. of this species is said to be widely cultivated in Russia to furnish spearmint type of herb and volatile oil (Russian Crisped Mint).

[8] Specimens not available to writer.

[9] Ruttle (11) indicates there are several different kinds of spearmint, as denoted by varying chromosome numbers. She recognizes that much of the spearmint of commerce is definitely not *M. spicata* but at the most a hybrid of this with other species.

[10] While the characteristic odor of spearmint is a convenient means of readily confirming identification, it may if carelessly used lead to errors in determination of *Mentha* specimens, hence is not a character ordinarily used by taxonomists.

[11] Heard used by workmen at Mentha, Mich. Mr. Stroud says: "The name 'Highland' was given to the plant by me in 1910 when I first had it in the experimental garden at Mentha and I doubt if that name has followed it beyond that vicinity although Dr. Ray Nelson has . . . decided to call it 'Mentha spicata var. Highland!' "

"A year or two previous to 1910 the late Mr. A. E. Beebe, on a vacation up in Wisconsin ran across the plant in a Scotchman's garden. This man had brought it from Scotland for culinary use. Mr. A. M. Todd secured a few roots from Beebe in 1910 and these I extended to several hundred acres in the course of a few years. Roots from these plantations were sent to St. Johns, Mich., and many other points so that now in 1947 I would conservatively estimate fully 75% of all spearmint produced in Michigan and Indiana is of the Scotch variety."

Guenther (16) also mentions the plant's introduction in 1910 into commercial cultivation. Apparently it increased in popularity rather slowly, however.[12] The most conservative current estimate is that about equal acreages are planted to each species (16). Harmer (68) recommends the Scotch "variety" for Michigan muck soils. An outstanding Michigan producer (17) estimates that 75% of the spearmint acreage in both that state and Indiana is composed of Scotch spearmint.

This impression that "true" spearmint is now much less common was also obtained from a field survey by the writer, who found only the Scotch type being grown in the vicinity of Charlotte, Potterville, and Lansing, Mich. Only at Mentha was a considerable acreage of *M. spicata* observed. Studies were made both of living plants in the field and (since it was late in the season) of the charges of exhausted hay (locally called "cheeses") brought from the still into the fields.

An important firm in Indiana (19) reports: "There is no single field of Native . . . Spearmint left any more in . . . States producing this oil. 98% is Scotch-type Spearmint and what little of the native or ordinary there is, same is mixed in the Scotch-type fields." Another important producer says: "With few exceptions, of small plots, Scottish is being raised entirely in Michigan. It produces a much larger yield than the native" (1948). In a study of northern Indiana mint fields, Smith and Robertson (20) said that in 1939, of 11 old settings of rhizomes, 3 were of "regular" spearmint and 8 of "Scotch," and of 9 new settings, 2 were "regular" and 7 "Scotch," showing here a net average of 75% "Scotch type" in the commercial plantings studied.

In the Michigan mint fields studied,[13] it was noted that the "Scotch" mint was set out in rows 3 feet apart with plants about one foot apart in the row, whereas native mint was placed much closer together in the row (about 4 inches between plants). The first year's growth is called "Row Spearmint," the second and later years' "Meadow Spearmint" (a result of the plant's spreading out between rows). As nearly as can be determined, Spearmint is not being produced in any quantity in Washington, Oregon, or other states, at present. Several years ago, one or more experimental plantings of Scotch mint are said to have been laid down in Oregon, but these seem not to have been continued (21).

While Scotch mint and common spearmint are readily separable by their botanical characters, the habit of these species is remarkably similar, a fact which has been noted frequently.[14] Hence, it is not surprising that some of the growers do not know which species they have, nor do the buyers always distinguish between the oils (22).

Distribution of *Mentha cardiaca*.—This species and the closely related *M. gentilis* (and varieties) are reported as cultivated in farm gardens in Central Europe (23), and also occur there wild though never spontaneously.[15] In England, *M. cardiaca* was found in "waste places" but "scarcely wild" (24), and is reported from 5 of the 71 "vice-counties" of Great Britain (25). It is grown in gardens, in Somersetshire, Wiltshire, Kent (26, 27), Surrey (28), and Oxfordshire (26, 29). The species is reported wild or quasi-wild from Yorkshire (30) and Surrey (28), Gower in South Wales (31), Durham, Oxfordshire, near Hertford, Middlesex, Norfolk, Leicestershire (32), and at such times has been referred to as a "denizen looking native but doubtfully indigenous." The species seems to have had a long history of cultivation (which may account for its superior properties), and is occasionally referred to as growing in medieval monastic gardens (28, 31). Fries (33) speaks of cultivation in Sweden and of spontaneous growth in a cultivated field at Lund up to 1823, when it yielded to cereal grains.

Similarly, in our country, *M. cardiaca* does not seem to have escaped widely from cultivation. It is not commonly treated in floras or collecting lists. Britton and Brown (34) report the range from Nova Scotia to New Jersey, Pennsylvania, and the District of Columbia, which can naturally now be extended to the states in which cultivated. Deam (35) notes finding the species growing spontaneously only once (in 1922) in Indiana, a state where many hundreds of acres of the species now receive cultivation. In Michigan, it was not mentioned by Beal in 1904 (36) (as would be expected at that early date), but was reported in 1923 from Fraser, Mich., and near by ("large patch") by Farwell (37), and again by Farwell in 1924 from Monroe Piers (6).

In the Medicinal Plant Garden of the University of Florida, as in many other gardens, this species has been grown as "spearmint," and appears to have originated there from plantings made in or about April, 1926, of what appeared in the records as "Scotch spearmint, *Mentha viridis* L." (Scotch spearmint was mentioned in the records again in 1927 and 1928.) These plants were reported to show good, actually profuse, growth, and to be spreading rapidly, doing especially well where the planting bordered the shade in the moister part of the Garden. Although not specified as "Scotch" in succeeding records, this species was grown in the Garden up to the present time, as proved by preserved herbarium specimens and photographs. It appears that in the course of the years, the "ordinary" variety of spearmint disappeared from the Garden, while the Scotch mint remained as a vigorous growth.

Examination of an herbarium specimen and of photographs of the "spearmint" of the Florida Garden, representative of the material used in the

[12] Thus, this species is not referred to in Bailey's Manual (18), published in 1924, presumably because it was not at that time an important commercial spearmint. The spearmint formerly a commercial crop in New York State was no doubt *M. spicata*.

[13] Visited among others were: Mentha, Mich.; James McCloud, Charlotte, Mich.; Muck Experimental Farm, near Lansing, Mich.

[14] However, it appears to the writer that generally speaking the herb of *M. Cardiaca* plants is a lighter green in color, often with reddish stems, and has strikingly bright pink flowers.

[15] The American vernacular name, Native Spearmint, is a misnomer, since *M. spicata* like most mints was introduced from Europe.

work of Christensen and Hiner (39), proves that this cultigen was *M. cardiaca*. Hence, the data reported in their paper relate not to *M. spicata* but to *M. cardiaca*. The same species was still under cultivation in the Florida Garden during 1940–1945.

NOMENCLATURE

Botanical Synonyms.—There is still some of the confusion in the species *Mentha sativa*, *M. gracilis*, *M. rubra*, and *M. gentilis*, that was manifest over a century ago (40, 41). What gives rise to this "unlimited confusion" (*grenzenlose Verwirrung*) (Ascherson) is in part the considerable tendency to variation of a number of important diagnostic characters, including plant height, leaf form, inflorescence type, form of calyx and calyx lobes (41).

There is no question that *M. cardiaca* is very closely related to the species *M. gentilis*, and Briquet's presentation of it as a variety of this species (71) may accurately reflect its true position. This classification is preferred by many systematists (the so-called "lumpers") who seek to reduce the number of recognized species and thus simplify and coordinate taxonomic names by subordinating many species to the rank of subspecies, variety, and other designations. Descriptions for *M. gentilis* as outlined by a number of writers, such as Small (42), Rydberg (43), Britton (44), *et al.*, appear inclusive of *M. cardiaca*. Both *M. gentilis* and *M. cardiaca*, as well as *M. sativa*, *M. gracilis*, and *M. rubra*, are generally believed to be hybrids of *M. arvensis* L. × < *M. spicata* L. By this means, both the verticillate inflorescence and the frequently appearing spearmint-type odor of these species receive rational explanation.

In addition to the four species named above, *M. cardiaca* is sometimes also confused with other verticillate species, such as *M. arvensis* or *M. canadensis*. For instance, Bentham includes *M. gentilis* as a synonym under *M. arvensis* (40, 12).[16]

Layman confusion with *M. spicata* has already been mentioned, but the plants' similar habit has also sometimes confused serious students.

M. cardiaca Gerard[17] ex Baker

Synonyms

M. gentilis L. var. (forma) *cardiaca* (Smith) Briq. (71)

M. gentilis L. subsp. 3 *cardiaca* Briq. (48)

Other botanical names which have been applied to this entity include:[18]

Sisymbrium sativum Matthiolus (10)
M. hortensis verticillata Ocimi odore Morison (47)
M. gracilis Smith var. β *Cardiaca* Smith (32)
M. cantalica Héribaud (49)
M. gracilis Sole (9)
M. gentilis L. β *glabrata* Fries

Vernacular Names.—The significance of the term "Scotch" as applied to this mint could not be established from the literature. The introduction of the plant from Scotland is referred to in an earlier paragraph and this may account for the name. The synonym was not, however, found in works on the flora of Scotland nor in the various exhaustive works on common names. Although reported apparently for Scotland by Sibbald (1684), Lightfoot (1777), Hooker (1821), *et al.*, the species is believed to have originated on the Continent. Another possible explanation for the common name lies in the occasional definition of "Scotch" as "to make incision in, notch, gash, score" (50). (Cf. "scotches," an agricultural term signifying scores or notches (51).) (Cf. also "scutch.") As noted elsewhere, the "notched" spike of Scotch mint is a ready distinction from the relatively regular "ungashed" spiciform inflorescence of true spearmint. Hence, Scotch mint may possibly have derived from "Scotched Mint." The name "spearmint" as used in association was applied no doubt from the odor and because of the sharpness of the leaves (52), as well, of course, as the resemblance in habit of the plants.

The name "Cardiac Mint" (9) and the specific name *cardiaca* originated most probably from the Latin "cardiacus" = heart- or stomach-affecting (53), because often used for treating "heartburn," gas on stomach and other similar ailments. (Cf. use of after-dinner mints.) This Latin term had as antecedent the Greek καρδία meaning heart, or the upper or cardiac orifice of the stomach, where the esophagus enters it (54). "Cardia" is also an English medical term having this latter meaning.

Other synonyms which have occurred at times are:[19]

Cardiaca (10)
Cardiaca Mentha (10)
Hartwurt (55)
Hart(-)woort (56)
Hart Mint (10, 55, 60); Hart Mynte (55)
Heart-Mint (56)
Her(t)zkraut (in "High Dutch") (10, 56)
Hertzmüntz (57)
Herbe de cueur (55)
Littleleaf Mint (58)[20]
Small-leaved Mint (34)[20]
Narrow-leaved Mint (24)
(True) Garden Mint (Somersetshire, Wiltshire, Kent) (8, 26)
Speare Mint (60)
"Mint" (Oxfordshire) (29)
Red Mint (62)
Red Sharp-leav'd Mint (?) (in Scotland) (Lightfoot)
Bushy Red Mint (?) (in Scotland) (Hooker)
Scotch Variety (or Type) of Spearmint (U. S. growers) (20)
"Highland Mint" (highly localized, Mich.)
Kukenbalsam (in German) (56)
Edelminze (in German) (23)
Edle Münze (in Swiss German) (63)
Edelmüntze (also applied to *M. gentilis*)[19]
Sisembrio Domestico (in Italian) (56)
Balsamita (in Italian) (56)
"Flora Coronata" (?) (55)

[16] To illustrate with one of many other examples, the inadequate description of *M. arvensis* by Wood (46) might well include both *M. gentilis* and *M. cardiaca*.

[17] The spelling as "Gerarde" occurs on the title page of the Herball (10), but although commonly adopted is undoubtedly in error (38).

[18] Acceptance is based on description or, preferably, where available, on herbarium specimens or figures. Many other synonyms which could not be checked have been compiled by various authors, e.g., Sole, Fries, Baker, *et al.*

[19] Some synonyms for *M. gentilis* are also applicable, such as: Gentile Mint; Herba (Menthae) Balsaminae; "Meadow Mint"; etc.

[20] So called because of the characteristic small (floral) leaves subtending the verticillasters.

CHARACTERISTICS

Botanical Characters of Scotch Mint.—*Mentha cardiaca* may be distinguished from other mint species which it resembles (*M. spicata*, *M. arvensis*, *M. canadensis*, *M. gentilis*, etc.) by the following characters (arranged in approximate order of importance): (1) Leaves short petiolate to petiolate throughout (in *M. spicata* sessile or very short petiolate).[21] This character alone is inadequate, since petiolate forms of *M. spicata* have been described (e.g., subsp. *exquisita* Bq. (48)). (2) Inflorescence axillary (subgenus *Verticillatae* L. = *Verticillastreae* Mvd. = *Axillares* Roch.), i.e., without the more or less continuous spiciform state seen in *M. spicata*, etc. (subgenus *Spicatae* L. = *Spicastreae* Bq.), or the capitate formation of *M. piperita*, etc. (subgenus *Capitatae* L.). (3) Axillary verticillasters are subtended by floral leaves (distinct from bracts[22]) from the top to the bottom of the inflorescence.[23] These floral leaves are however considerably smaller than the lower cauline foliage leaves and are not of the relatively large dimensions seen in *M. arvensis* and *M. gentilis*, in which there is only slight reduction in leaf size as one proceeds up the stem and passes from the vegetative to the flowering part of the axis.[24] In *M. cardiaca*, the reduced leaves extend down the axis for a considerable distance; in fact, the largest leaf found (subtending a lowermost verticillaster) measured only 35 mm. in total length. The lower verticillasters are rather widely separated. (4) The base of the calyx and the entire pedicel are glabrous and not pubescent as in *M. arvensis*, *M. canadensis*, etc.[21] (Fig. 3, *H*). (5) Calyx and corolla are both glabrous internally. (6) Leaves and stems of *M. spicata* and of *M. gentilis* sometimes are glabrous or nearly so, whereas for *M. cardiaca* these organs are typically quite hairy, but not as densely clothed as generally in *M. arvensis*, etc. (7) Foliage leaves are typically broader, less oblong than in *M. spicata*, but are not as broad as usual for *M. gentilis*, *M. arvensis*, etc.; and are much less obtuse than in *M. gentilis*. (8) Foliage leaves are strongly and sharply serrate, somewhat similar to those of *M. gentilis*, but radically different from those of *M. spicata* (Fig. 3, *B*, *P*). (9) Calyx tubular-campanulate (Fig. 3, *G*, *H*), not campanulate as in *M. gentilis* and *M. spicata* nor tubular as in *M. arvensis*. (10) *M. cardiaca* grows commonly 1.2 to 1.4 m. high and hence is taller than *M. spicata*, at least in cultivation. (11) Foliage is colored a light and bright shade of green. Leaves of *M. gentilis* are characteristically much darker, often distinctively marked on the upper side by whitish areas adjacent to midrib and veins.[25] (12) Twelve to thirteen veins traverse the calyx of *M. cardiaca* (10 in *M. arvensis*, *M. gentilis*). (13) The flower in *M. cardiaca* is decidedly larger than in *M. spicata*, *M. gentilis*, etc., and approaches the flower size in *M. arvensis*. (14) The calyx lobes of *M. cardiaca* are relatively much shorter than the tube as compared with *M. spicata* (Fig. 3, *H*).

Petioles.—*Mentha spicata* is commonly stated to have sessile to subsessile leaves. Topitz (5) describes the leaves of this species and varieties as sessile, subsessile, short petiolate, and subpetiolate, although these last three terms would seem almost identical. Fassett (66) and Sun (67) speak of petioles 0 to 2 mm. in length. Baker (64), the post-Linnaean author of the species, and some other writers, have described the leaves of *M. Cardiaca* as sessile or with the lowest very short-stalked, whereas the writer's observations indicated leaves with medium long petioles throughout. The figure of Gerard (56), who was cited by Baker as the author, indicates definite petioling, and his description speaks of "long leaues . . . in [on] stalks."

To check these points, a study was made of hundreds of herbarium specimens of these two species. Following a careful check on the identity of each specimen, the petioles were examined[26] and a single maximum length was recorded (to the nearest millimeter) for each specimen[27] (Table I).

TABLE I.—DISTRIBUTION OF PETIOLE LENGTHS

Mentha spicata	Length, Mm.	No Specimens
Av. = 1.1 mm.	0	85
	1	112
	2	94
	3	16
	Total	307
Mentha cardiaca		
Av. = 6.4 mm.	2	3
	3	8
	4	16
	5	12
	6	20
	7	20
	8	16
	9	8
	10	9
	11	4
	12	2
	14	2
	Total	120

[21] Malinvaud recognized characters (1, 4) as "precise and constant" for *M. gentilis* (under which he recognized Cardiac Mint as a variety).

[22] Typical bracts differ from these floral leaves in their scale-like conformation: narrow, elongate, of dark reddish color, provided with two lateral lobes similar in appearance and only slightly smaller.

[23] In *M. spicata*, the lowest verticillaster alone is sometimes subtended by a pair of leaves in the fashion of *M. cardiaca*.

[24] In *M. gentilis*, however, considerable reduction in floral leaf size occurs usually within 1 to 3 cm. of the stem tip. There is sufficient variation on this point, however, to make it easy to confuse the species unless care is taken. Thus, in Britton and Brown (34), the genus diagnosis properly separates *M. cardiaca* from *M. gentilis*, etc., but under the specific description, *M. gentilis* is contradictorily stated to have upper leaves "sometimes much smaller than the lower," in this way nullifying the distinction in part. In *M. gentilis* the verticillasters are carried in the axils of very large leaves (sometimes the largest on the plant, along the center of stem) and down the stem for considerable distances (sometimes over 30 cm.). (In Baker's plate of *M. cardiaca* (64), some "whorls, such as the eighth down, are in the axils of rather larger leaves than found in the writer's observations.) In atypical specimens of *M. gentilis* (hybrids or varieties) where the verticillasters were subtended above by small, below by large leaves, it was found that the floral leaves have longer petioles than in *M. cardiaca*, hence extend farther out. There appears to be a fairly well-defined gradation in size of floral leaves, starting with *M. spicata*, which has diminutive leaves bracketing the lowermost verticillasters. Then come *M. cardiaca*, with pronounced leaves, and increasing in prominence, *M. gentilis*, then *M. arvensis*.

[25] This species is said to show an extreme variegated form known as "Golden Apple Mint" (11).

[26] Petiolar length varies within limits according to the amount of moisture available to the plant (61, 65). Although considerable variability in petiolar length is manifest within these species, yet each is still distinctive with little overlapping of limits.

[27] It has been said that leaves taken from the main stem and not from the branches are most typical and should alone be considered. As far as petiolar length, this could not be confirmed. Also, up to about the center of the stem, no recession in petiolar length could be noted as compared with the lowermost leaves.

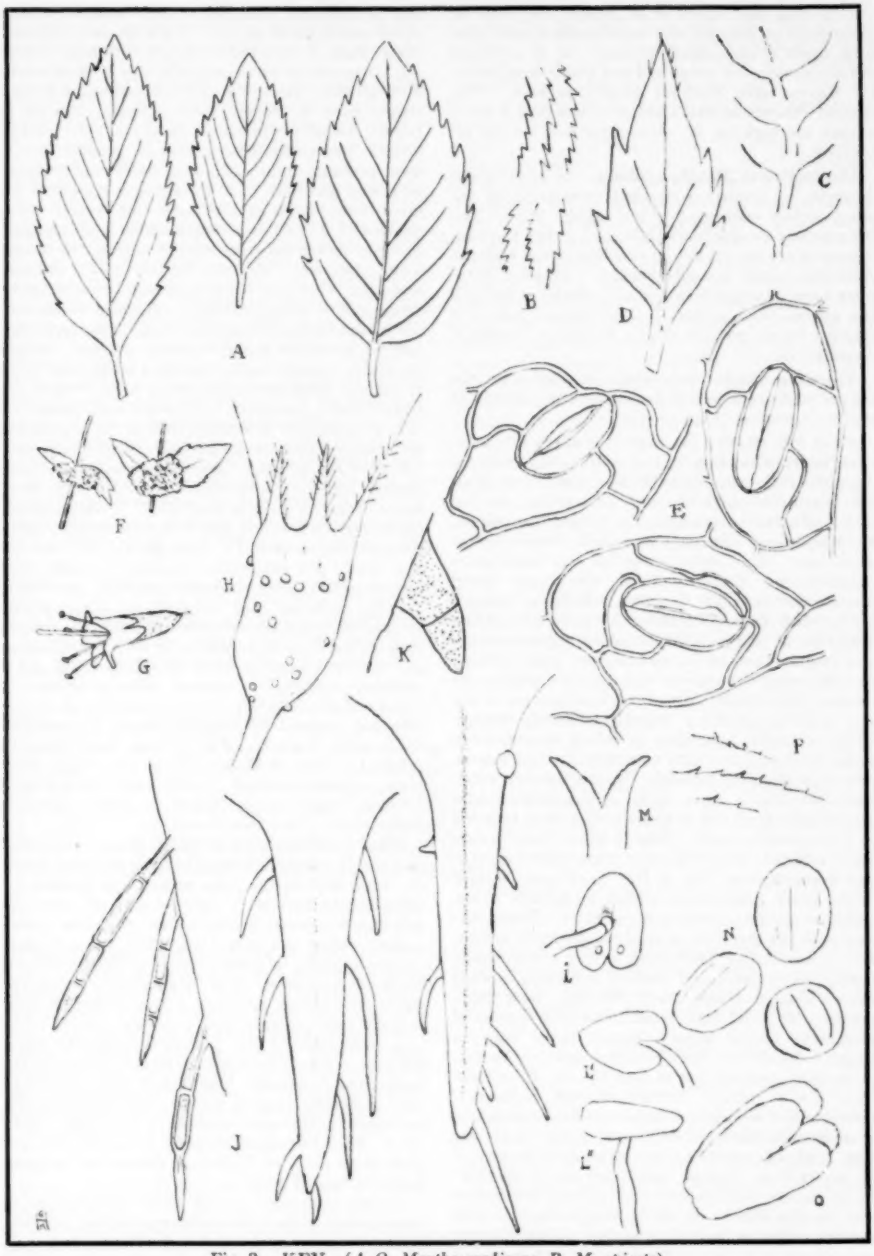

Fig. 3.—KEY (*A–O, Mentha cardiaca; P, M. spicata*)

A, tracings of 3 foliage leaves; *B*, margins of foliage leaves; *C*, bases of foliage leaves; *D*, floral leaf; *E*, stomata from stem epidermis; *F*, verticillasters; *G*, flower with exerted stamens and pistil; *H*, calyx; *J*, calyx lobes with uniserial mechanical hairs; *K*, conical hair on calyx; *L*, stamen from below; *L'*, stamen from above; *L''*, stamen from side; *M*, apex of pistil; *N*, pollen grains; *O*, fruit; *P*, *M. spicata*, margin of foliage leaf.

Enlarged: *D, G, H, L, L', L'', O.* Much enlarged: *E, J, K, M, N.* Others: approx. natural size.

It is clear that leaves of *M. spicata* are sessile or practically so, with petioles occasionally up to 3 mm. long, hence at most short petiolate. In *M. cardiaca*, petioles mostly run between 4 and 8 mm. long, hence its leaves have medium length petioles. The present Pharmacopœial allowance (less than 5 mm.) is then too high for *M. spicata*, but too low for *M. cardiaca*.

Description of *Mentha cardiaca*.—It was thought desirable to present a detailed description of the plant as now cultivated in the Middle West. The descriptions presently available are either too condensed or are at variance to a certain extent with the American plant, possibly because European botanists have distinguished several varieties or forms of the species (such as var. 1, 2, of Baker; var. *α*, *β* Smith; forma *gratiosa* Topitz, f. *geniata* Topitz, f. *Sagorskii* Bq.).

The data which follow were compiled from the careful study of scores of specimens, both cultivated and wild-growing (Figs. 2, 3).

Plant 9 to 10 to 14 dm. high.—*Stem* erect, sturdy, with many ascending and spreading branches toward the top; retrorse-pubescent, most marked on the vegetative parts of stem, near nodes, and on angles of stem; often glabrous toward base, somewhat scabrous; frequently reddish, deepening toward base. Internodes 2.6–*4*–5.7 cm. long below inflorescence. *Leaves* petioled, the upper (floral leaves) short-petiolate (2–4 mm.), the lower (foliage) leaves with distinct petioles 2–*6.4*–14 mm. long. Serration of margin sharper, more regular, closer, and coarser than in *M. spicata*, the teeth pointed forward, with the exterior side nearly straight, the interior side usually curved, each measuring 4–*5*–6 mm. x 1–*1.4*–1.8 mm.; sometimes doubly serrate. Shape of lamina lanceolate to oblong-lanceolate, to ovate to ovate-lanceolate to elliptical; leaf lamina relatively elongate typically (length/breadth ratio: 2.05–*2.29*–2.72). Apex acute to acuminate; base usually narrowed, but in larger leaves often rounded or even obtuse, entire (Fig. 3, *C*). Floral leaves much reduced, 2 to 3 times or more exceeding axillary floral clusters (Fig. 3, *D*). Leaf upper surface bright green (sometimes reddish on midrib, veins, and near margin), lower surface lighter. Upper surface generally glabrous, or occasionally hairs on midrib; lower surface pubescent, with scattered hairs mostly along veins and margin, especially toward base. Foliage leaves 35–*57*–88 mm. long (incl. petiole), 16–*25*–37 mm. broad, with 5 to 6 pairs of prominent lateral veins. Floral leaves vary in length 4–*20*–35 mm. (incl. petioles), and 2–*9*–18 mm. in breadth (typical size of leaf 15 mm. from stem apex, 5 x 13 mm.). Petioles densely pubescent. Inflorescence verticillate (*status axillaris* with leaves in *status subbracteosus*), with main axis 7–*12*–30 cm. long, with the greatest concentration of flowers in the upper 8 cm. approx., consisting of a series of 6–*13*–19 mostly well-separated subglobose verticillasters (pseudo-whorls) on the main axis, together with smaller series on lateral branches. Verticillasters are subtended in axils of floral leaves, and separated by from 3–*25*–42 mm.; internode length increases toward the lower part of the inflorescence. A few uppermost axils on stem and branches are mostly without flowers. The succession of verticillasters is ordinarily terminated below at the first pair of floral leaves standing above the origin of the first pair of flowering branches. These lateral branches never exceed the main axis. Verticillasters relatively stout, diam. 5–*11*–14 mm., height 6–8 mm. (Fig. 3, *F*). Diameter of verticillaster to tips of floral leaves 8–*29*–50 mm.; ratio (length floral leaf/length flower) ranges from 2 (upper) to 5 (lower). Fifteen to twenty flowers per peduncle, or 30 to 40 per verticillaster. Bracteoles 2 per peduncle, subulate-linear, short petiolate, lightly ciliate on midrib and margins, otherwise glabrous; 3.7–*4.6*–5.2 mm. long, 0.2–0.6 mm. wide, considerably exceeding flower. Peduncles 1.2–*1.7*–2.7 mm. long, however much elongate in lower flower clusters, where found up to 6 mm. long, glabrous. Pedicels 0.6–0.7 mm., reddish, glabrous, with occasional glands. *Calyx* tubular-campanulate to campanulate; glabrous inside and out except for teeth, which are hispid, also have unicellular glandular hairs; exterior of calyx thickly glandular, glands mostly localized along both sides of the five most prominent ribs; 12–13-veined; in flower, calyx measures 2.0–*2.4*–2.7 mm. long, 0.8 mm. in diameter; persistent in fruit; green before, brownish red after anthesis. Tube slightly furrowed, 1.3–*1.6*–1.8 mm. long. Teeth linear-subulate, some slightly larger than others, 0.7–*1.0*–1.3 mm. long, hence decidedly shorter than tube. *Corolla* 4-lobed, the broadest upper lobe notched, lobes obtuse; pinkish-lavender, sometimes light pink; 3.7–*4.2*–4.4 mm. long; 2.4 mm. max. diameter; corolla tube exceeds calyx; glabrous inside and out; glandular. *Stamens* included and exserted on same plant; heterostyly noted (in some flowers, exserted stamens with included pistil, in others the reverse; occasionally observed exserted pistil with 2 exserted and 2 included stamens); anthers dehisce introrsely, measure 0.35 x 0.50 mm. (av.). Pollen ovoid or subspherical, grooved, 25–28 mμ av. length, sometimes in 3's (1 large, 2 small). *Pistil* 6 mm. long, typically exserted; style 0.45 mm. long, 0.12 mm. thick (av.); stigmata reflexed. *Nutlets* ovoid, dark brown, 0.7 mm. long, warty, bristly at apex. *Palisade Ratio:* 6.5.[29] *Vein Islet Count:* 13.

Other Characteristics of Scotch Mint.—Comparative tests made by crushing the fresh herb or rubbing the dried herb in the palm reveal little qualitative difference in odor of *M. spicata* and *M. cardiaca*, although it is generally obvious that the latter species is much richer in aroma. Guenther (16) says some customers prefer oil from "common," others oil from "Scotch" spearmint. A leading producer in Indiana states that Scotch mint has a 20% higher oil yield and has more of the "minty" aroma, at the same time with less of the "biting" (pungent) taste. Oil from mixed fields is sold to buyers preferring the more pungent article. The "much higher" oil content of Scotch mint is widely confirmed (e.g., by outstanding Michigan producers), and some report up to 50% increased oil yield from this species. This is naturally an important reason for its popularity with mint growers.

Various operators say the chief disadvantage of Scotch mint is its lesser hardiness to cold weather, its lower resistance to insect and fungal attack (e.g., it is rather vulnerable to the *Verticillium* wilt which has caused such havoc in mid-western mint fields), and its need of being more frequently replanted.

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[29] Cf. figures for *M. spicata:* 6.0–*6.2*–6.8 (59).

Countering these are such stated advantages as Scotch mint's earlier maturity, larger size, bigger yields, and apparent better thriftiness in warmer climates (such as in Florida).

As far as the writer is aware, no comparative studies have yet been made on the oils of *M. spicata* and *M. cardiaca*. Data in the literature for American "spearmint oil" may be based on either species or on both in admixture.

It would seem warranted to resurvey standards for spearmint oil on the basis of botanical origins. Analyses which have appeared in recent years (39, 69, 70) show a far wider latitude of constants and of carvone percentage than required in the official monograph for the oil. A systematic study of the oils is definitely indicated and might reasonably be expected to show a quite distinctive range of values for each species. Such a study is to be undertaken by the author.

The shifting standards for spearmint oil in the U. S. P. over the last sixty-five years are indicated in Table II. Note especially the alterations in optical rotation and the increase in carvone percentage between 1905 and 1936. From what has been said as to its increasing popularity with producers, it would be a good surmise from these data that Scotch mint oil has probably a higher levo-rotation and carvone content than true spearmint oil.

It is therefore recommended that the official monographs for both the herb and the oil be amended so as to include the additional species "*Mentha cardiaca* Gerard ex Baker," the synonym "Scotch mint," and changes as follows in "Description" (of Herb) (additions are set within quotation marks):

Leaves . . . ovate-lanceolate "to ovate, oblong-lanceolate, elliptic" . . . 3 to "9 cm." in length, margin "sharply" serrate. Surfaces "glabrous (*M. spicata*) or" under surface with a few hairs on the veins "(*M. cardiaca*)," nearly sessile "(*M. spicata*)" or "with petiole less than 15 mm. in length (*M. cardiaca*)." Stems . . . green "but generally dusky red or purplish." Verticillasters . . . "spiciform (*M. spicata*) or in separated axillary clusters, each subtended by a pair of floral leaves resembling the foliage leaves in appearance but smaller (*M. cardiaca*); the spiciform inflorescence 3 to 9 mm. (*M. spicata*) or 5 to 14 mm. (*M. cardiaca*) in diameter, and in fruit up to 10 cm. (*M. spicata*) or 15 cm. (*M. cardiaca*) in length (occasionally up to 30 cm. in latter)." Calyx campanulate "(*M. spicata*) to campanulate-tubular (*M. cardiaca*), subequally" 5-toothed, . . "glabrous or nearly so throughout (*M. spicata*) or glabrous below with hispid teeth (*M. cardiaca*)," green "(*M. spicata*)" to purplish red "(*M. cardiaca*)." Corolla . . . orange "to pinkish to lilac and purple . . . " its tube shorter than the calyx "(*M. spicata*) or longer (*M. cardiaca*)." Style . . . "exserted." Nutlets . . . "minutely grained (*M. spicata*), warty (*M. cardiaca*), dark brown."

TABLE II.—OFFICIAL STANDARDS FOR SPEARMINT OIL OF THE UNITED STATES PHARMACOPŒIA, 1880–1947

Revision (Publication Date)	d_{25}	$(\alpha)_D$	n_{25}	Carvone, %
1880[a] (1882)	0.900 approx.	...	...	...
1890 (1894)	0.930–0.940	...	...	...
VIII (1905)	0.914–0.934	−35°–48°	...	...
IX (1916)	0.917–0.934	−38°–55°	...	NLT 43
X (1926)	0.917–0.934	−38°–56°	1.4820–1.4900	NLT 43
XI (1936)	0.917–0.934	−48°–59°	1.4820–1.4900	NLT 50
XII (1942)	0.917–0.934	−48°–59°	1.4820–1.4900	NLT 50
XIII (1947)	0.917–0.934	−48°–59°	1.4840–1.4910	NLT 50

[a] No standards at all given in U. S. P. 1870 and previous revisions.

Uses of Scotch Mint.—*Mentha cardiaca* has been used in the same manner as true spearmint since apparently as early as the time of Parkinson (1640) (60) or possibly still earlier. The old herbalists considered the plant superior to spearmint as a cardiac ("heart" remedy, really heartburn remedy)[39] and stomachic, but inferior as a febrifuge. Jackson (27) experimented with this species in mint sauce and decided it had a poorer flavor than *M. spicata*.

DISCUSSION

It has been plainly shown that Scotch mint has been and is now an important source of spearmint oil. It also constitutes a portion of the spearmint herb of commerce, since a study of commercial lots of "spearmint herb" by the author showed that Scotch mint occurs very often, both alone and in admixture with *M. spicata*.

[39] The herb has, of course, no authentic reputation as a heart stimulant, as presumed by some (59).

SUMMARY

1. A study has been made of wild-growing and cultivated spearmints of the United States, past and present, in the field as well as from herbarium specimens and textual descriptions, and it has been shown that, in addition to true spearmint, an increasingly large proportion of the commercial herb and oil derives from "Scotch spearmint."

2. Wrongly called a "form" of spearmint, "Scotch mint" is a distinct entity which belongs neither to the species *Mentha spicata* L. nor even to the respective subgenus *Spicatae* L., but to a

different species and subgenus of *Mentha*, viz., *M. cardiaca* Gerard ex Baker of subgenus *Verticillatae* L.

3. A fairly complete description of *M. cardiaca*, and several means of distinguishing it from *M. spicata* (True Spearmint) and other *Mentha* species, are presented.

4. The reported variation of spearmint oil in constitution and assay may be explained perhaps by differences between Scotch mint oil and that of true spearmint, on which latter the earliest standards were based. Studies on the respective oils of the two species are to be undertaken.

5. On the basis of a study of many authenticated specimens of both "spearmints," suggestions are advanced for bringing the official monographs up to date.

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Chemical Composition of Gum Turpentines of Pines: A Report on *Pinus strobus, P. cembra, P. taeda, P. radiata,* and *P. virginiana**

By N. T. MIROV, T. H. WANG,† and A. J. HAAGEN-SMIT†

The physical and chemical properties of turpentine obtained from five species of pines have been determined and are reported in this article.

SOME THREE years ago, in cooperation with the California Institute of Technology, the California Forest and Range Experiment Station began investigating the chemical properties of turpentines from different species of the genus *Pinus*. The primary interest of the Experiment Station in this project was to study the nature of the inheritance of biochemical characters of the genus *Pinus* to supplement other genetic research of the Institute of Forest Genetics. Reasons for selecting turpentines for this purpose and application of the information obtained to the problems of forest genetics are fully discussed in a recent article by one of the authors (1). The interest of the California Institute of Technology—more specifically of the laboratory of bio-organic chemistry—in this project is that of exploring the little-known field of chemical composition of turpentines of the genus *Pinus*.

The results reported in this paper deal with turpentine composition of five species of pines: *Pinus strobus, P. cembra, P. taeda, P. radiata,* and *P. virginiana*. Oleoresin samples used in this project were either obtained by members of the Institute of Forest Genetics or received through the courtesy of different persons as indicated in the text. Turpentine was distilled from the oleoresin at the Institute of Forest Genetics Laboratory, Placerville, Calif., with the assistance of E. F. Kimbrough, and physical characteristics of the turpentine were determined and a trial fractionation was made. The main fractionation and identification of the components were done at the Department of Bio-organic Chemistry, California Institute of Technology at Pasadena, Calif.

This article is the third one written jointly by California Institute of Technology and California Forest and Range Experiment Station. More articles are to follow.

PINUS STROBUS

Pinus strobus (L.) is commonly known as eastern white pine. Its range extends from the White Bay region of Newfoundland to northern Ontario, to southern Manitoba, southward through northern and eastern Minnesota, to Iowa, and northern Illinois and Michigan. Once it was abundant as far south (along the Allegheny Mountains) as northern Georgia. Its variety *Chiapensis* occurs in the mountains of southern Mexico (States of Chiapas, Oaxaca, and Vera Cruz).

Oleoresin for the present analysis was collected by staff members of Lake States Forest Experiment Station. The oleoresin was collected in the northern part of Minnesota (Chippewa National Forest) from fourteen 100-year-old trees about 20 inches in diameter. Altogether 3800 Gm. of oleoresin was received.

The rosin acids did not crystallize as readily as in oleoresin of the yellow pines. On the contrary, a honey-like consistency was retained in storage for a long time. It should be noted that oleoresins of all white, or Haploxylon, pines have this tendency to remain liquid for a long time, while oleoresins of the majority of Diploxylon pines usually become sugary and hard very soon after collecting. Apparently there is a definite difference in the composition of the rosin between the Haploxylon and the Diploxylon groups of the genus. Only a few species of pines have been investigated as to the compositions of their rosins. Lombard (2) reported that oleoresin of white (Haploxylon or soft) pines is rich in abietic acid; oleoresin of hard (Diploxylon) pines rarely contains it, but is characterized by the presence of lævo-pimaric acid.

For the present analysis, the turpentine was distilled from the oleoresin under reduced pressure; toward the end of the distillation the temperature inside the flask reached 205°, and the pressure was reduced to 0.05 mm. The turpentine obtained amounted to 25% of the oleoresin.

The turpentine had the following characteristics:

Density, d_4^{25}	0.8611
Index of refraction, $n_D^{20.8}$	1.4714
Specific rotation, $[\alpha]_D^{23}$	−0.75°

The data obtained in fractional distillations are assembled in Table I. Analytical data are given in Table II.

The main part of the oil consists of *dl*-α-pinene (75%) and β-pinene (15%) as shown by the preparations of nitrosochloride (m. p. 106–107°) and nopinic acid (m. p. 125°), respectively. This is in harmony with the physical constants given in Table I and the

* Received May 15, 1949, from the California Forest and Range Experiment Station, which is maintained by the Forest Service, U. S. Department of Agriculture, in cooperation with the University of California, Berkeley.

† Department of Bio-organic Chemistry, Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena.

analytical data in Table II. Tests for Δ^3-carene were negative. The higher boiling fractions, 12–15 of the oil contain, as seen from Table II, terpene alcohols, terpene ketones, mixed with terpene hydrocarbons.

Fraction 16 consists solely of a sesquiterpene hydrocarbon (0.3%). After distillation over Na the physical constants of the fraction were: d_4^{25}, 0.9131; n_D^{25}, 1.4913; boiling point 110–115° at 27 mm. Molecular refraction for this hydrocarbon ($C_{15}H_{24}$) was found to be 64.83; calculated for a sesquiterpene ($C_{15}H_{24}$) with one double bond, it would be 64.40. This points to the presence of a tricyclic sesquiterpene.

Tchermark of the University of Vienna. A sample amounting to 1571 Gm. was collected during the summer of 1947 from 30 trees in "der Forstverwaltung Paal bei Murau, Obersteiermark, Austria," at an elevation of 5400 to 5600 feet. The oleoresin was rather liquid but with some rosin acids crystallized out.

From 1571 Gm. of the oleoresin, 320 Gm. (or 20.4%) of turpentine was obtained. The turpentine had the following properties:

Density, d_4^{25}	0.8522
Index of refraction, $n_D^{25.5}$	1.4654
Specific rotation, $[\alpha]_D$	+13.83°

TABLE I.—FRACTIONAL DISTILLATION OF 550 GM. OF *Pinus strobus*

Fraction	Distillation Range: Pressure, Mm.	Distillation Range: Temperature, °C.	Distillate, %	Density, d_4^{25}	Index of refraction, n_D^{25}	Specific rotation $[\alpha]_D^{25}$
1	740	154–155	1.0	....	....	.....
2	740	155–156	53.6	0.8590	1.4667	+1.85°
3	740	156–157	18.7	....	....	.....
4	740	157–158	1.3	....	....	.....
5	740	158–159	1.5	....	....	.....
6	740	159–160	2.4	....	....	.....
7	740	160–161	5.4	0.8610	1.4707	−10.23°
8	740	161–162	4.3	....	....	.....
9	740	162–164	2.9	0.8590	1.4738	−6.93°
10	740	164–165	1.6	0.8534	1.4740	−2.89°
11	740	165–170	0.8	0.8611	1.4812	.....
12	1.0	60– 70	0.5	0.8732	1.4840	.....
13	1.0	70– 80	0.6	0.9121	1.4884	.....
14	1.0	80– 90	0.2	0.9281	1.4890	.....
15	1.0	90– 94	0.8	0.9223	1.4915	.....
16	1.0	94–100	0.3	0.9264	1.4974	.....
Residue and loss	...	.....	3.7	....	....	.....

TABLE II.—ANALYTICAL DATA OF FRACTIONS[a]

Fraction	Analysis: Carbon, %	Analysis: Hydrogen, %	Determined by Zerewitinoff[b] Method: Active H	Determined by Zerewitinoff[b] Method: Carbonyl Group
3	87.94	12.28	.....	.....
6	88.35	11.79	.....	.....
12	87.30	11.81	0	0.2 mol.
15	84.24	11.57	0.2 mol.	0.4 mol.
16	88.06	12.11	.....	.....

[a] Calculated: for $C_{15}H_{24}$ or $C_{15}H_{26}$: C = 88.17%; H = 11.83%; for $C_{15}H_{24}O$: C = 78.91%; H = 10.58%.

[b] Soltys, Arnulf, "Die micro analytische Bestimmung der aktiven Wasserstoffes nach L. Tschugaeff und Th. Zerewitinoff," *Mikrochemie*, **20**, 107(1936).

The fractionation was carried out at ordinary pressure in the usual manner. The fractionation data and physical constants are given in Table III.

From fraction 3 a nitrosochloride was prepared; its melting point was 106–107°, identical with that of α-pinene. After oxidation of fraction 10 with $KMnO_4$, nopinic acid was isolated with a melting point of 126–127°, identical with that obtained from β-pinene. Tests for dipentene on fraction 13 were negative since no tetrabromide could be separated. Tests for Δ^3-carene were negative. The main constituents of the oil are, therefore, α-pinene, 85%, and β-pinene, 9%.

The amounts of higher-boiling constituents show a small maximum, probably in the boiling range 173–175°, at 742 mm. This fraction contains mostly mixtures of isomerization and oxidation products of pinene from which no crystalline derivative could be prepared.

Fraction 15 dehydrogenated with sulfur did not give cadalene or other naphthalene hydrocarbon.

PINUS CEMBRA

Swiss stone pine (*Pinus cembra*) is a European pine growing at high altitudes in the Alps, from Savoy to the Carpathians. Some botanists maintain that *P. sibirica* of the vast plains and mountains of northeastern Russia and of Siberia is botanically identical with *P. cembra*, and that scrubby *P. pumila* of northeastern Siberia and northern Japan is a variety of *P. cembra*. *Pinus pumila* turpentine has never been analyzed, but that obtained from *P. sibirica* was partly analyzed by Schkateloff (3). A sample of old, oxidized oleoresin yielded only 6% of turpentine which was found to be dextrorotatory, $[\alpha]_D = +14°$, having density, $d_4^{15} = 0.865$; 20% of it boiled between 155° and 156°.

Oleoresin for the present analysis was obtained through the assistance of Professor Dr. H. Knüchel, of Zurich, Switzerland, and Professor Dr. Leo

PINUS TAEDA

Loblolly pine (*Pinus taeda*) occurs in the southeastern United States from New Jersey to central Florida; west to eastern Texas, northward into southwestern Oklahoma, southwestern Arkansas, and the southern border of middle and west Tennessee.

Loblolly pine turpentine had been previously obtained by Charles H. Herty. His sample was dextrorotatory (+46°); its density, 0.8525, and

index of refraction, 1.4700; fractionation yielded the following results:

9.3% came over between 160–165°
38.0% came over between 165–167°
37.5% came over between 167–172°
12.5% came over between 172–180°
2.7% residue boiled above 180°

From these results it was concluded that the volatile oil from loblolly pine is a mixture of pinene and limonene, with the latter probably in excess. No identification of the components was made.

Oleoresin of loblolly pine used in the present work was sent by Mr. Keith Dorman, of Southeastern Forest Experiment Station, Lake City, Fla. Upon distillation under 0.05 mm., 3818 Gm. of oleoresin yielded 710 Gm. or 18.6% of turpentine. The relatively low yield may be explained by the loss of some volatile oil from a jar broken in transit.

The turpentine had the following constants:

d_4^{23}	0.8570
Index of refraction, $n_D^{23.5}$	1.4675
Specific rotation, $[\alpha]_D$	+20.17°

A sample of loblolly pine turpentine oil was first distilled into three fractions—the low, medium, and high boiling—and each of these fractions was further fractionated. The combined results of these fractionations and the physical properties of the fractions are given in Table IV.

The data in Table IV show that the oil consists largely of *d*-α-pinene, 85%, and *l*-β-pinene, 12%. The nitrosochloride of fraction 5 melts at 106–107°, and gives no depression when mixed with an authentic sample of *d*-α-pinene nitrosochloride. The physical properties of fractions 8 and 9 indicate the presence of *l*-β-pinene. A sample of fraction 9 was oxidized with an aqueous solution of potassium permanganate and sodium hydroxide, and nopinic acid was obtained. The acid after recrystallization melted at 125–125.5°, while the melting point of nopinic acid as recorded in literature is 125°.

The boiling point range, 168–172°, of fractions 11 and 12 suggested that Δ^3-carene might be present, but attempts to prepare Δ^3-carene nitrosate from these fractions failed.

While fraction 13 gave carbon hydrogen values showing the presence of $C_{10}H_{16}$ hydrocarbon, tests for limonene were negative. In a test with fuming sulfuric acid no saturated hydrocarbons could be isolated.

PINUS RADIATA

Pinus radiata (D. Don) is the Monterey pine of California. It grows naturally in a few places on the

TABLE III.—FRACTIONAL DISTILLATION OF THE VOLATILE OIL FROM *Pinus cembra*

Fraction	Distillation Range: Pressure, Mm.	Distillation Range: Temperature, °C.	Distillate, %	Density, $d_4^{20°}$	Index of Refraction, n_D^{20}	Specific Rotation, $[\alpha]_D^{20}$
1	742	100–154	0.8	....	....	.....
2	742	154–155	25.8	....	....	.....
3	742	155–156	45.0	0.8539	1.4640	+18.38°
4	742	156–157	6.0	0.8540	1.4639	+17.63°
5	742	157–158	3.5	....	....	.....
6	742	158–159	3.5	....	....	.....
7	742	159–160	1.0	0.8557	1.4668	+11.10°
8	742	160–162	1.0	....	1.4706	+10.34°
9	742	162–164	2.5	0.8580	1.4690	+ 7.75°
10	742	164–166	1.5	....	....	.....
11	742	166–168	2.5	0.8575	1.4744	+ 2.92°
12	742	168–173	0.5	....	....	.....
13	742	173–175	1.3	0.8684	1.4790	+ 3.10°
14	742	175–180	0.5	....	....	.....
15	742	.....	3.4	....	....	.....
Residue	...	.....	0.2	....	....	.....

TABLE IV.—FRACTIONAL DISTILLATION OF THE VOLATILE OIL FROM *Pinus taeda*.

Fraction	Distillation Range: Pressure, Mm.	Distillation Range: Temperature, °C.	Distillate, %	Density, d_4^{25}	Index of Refraction, n_D^{25}	Specific Rotation, $[\alpha]_D^{25}$
1	740	100–144	0.2	0.8626	1.4576	.....
2	740	144–152	0.40	0.8556	1.4639	.....
3	740	152–154	1.60	0.8546	1.4645	+33.0°
4	740	154–156	16.50	0.8548	1.4650	+31.67°
5	740	156–158	41.60	0.8548	1.4669	+23.74°
6	740	158–160	25.20	0.8555	1.4674	+15.56°
7	740	160–162	1.90	0.8556	1.4691	+12.36°
8	740	162–164	3.8	0.8592	1.4725	− 3.38°
9	740	164–166	2.6	0.8608	1.4743	− 5.52°
10	740	166–168	1.2	0.8605	1.4822	.....
11	740	168–170	1.2	0.8686	1.4790	.....
12	740	170–176	1.7	0.8686	1.4750	.....
13	740	176–196	1.0	0.8570	1.4750	.....
14	740	196–208	0.5	0.9455	1.5016	.....
Residue	...	.....	0.6	....	....	.....

coast south of San Francisco, particularly on the Monterey peninsula and some near-by islands. It has been planted very extensively all over the warmer parts of the world, and in Australia, where it is known as *Pinus insignis*, has become a commercial source of lumber. Turpentine of this pine has been previously investigated in Russia, France, and New Zealand, with the results as given in Table V.

The oleoresin for the present analysis was obtained from planted trees about twenty years of age and 6 to 8 inches in diameter[1] growing in the Eddy Arboretum of the Institute of Forest Genetics. It was collected from the middle of July to the end of August; the yield was extremely small. The oleoresin solidified in the receptacles into a hard mass.

Upon distillation of about 1700 Gm. of oleoresin, 285 Gm. of turpentine was obtained, which amounted to only 16.8%. The turpentine had the following physical constants:

Density, d_4^{25}	0.8596
Index of refraction, n_D^{25}	1.4727
Specific rotation, $[\alpha]_D$	−5.85°

The oil was fractionated in the usual manner, the results of which are listed in Table VI.

Fraction 3 was tested for α-pinene with amyl nitrite and hydrochloric acid. The crystalline nitrosochloride melted at 106–107° and gave no depression with an authentic sample of α-pinene nitrosochloride.

Fraction 9 was oxidized and nopinic acid, melting point 126–127°, was obtained.

TABLE V

Author	Reference	Composition	Physical Constants: Density	Physical Constants: Index of Refraction	Physical Constants: Specific Rotation
Barraud, Marcelle	"L'état de nos connaissances sur la composition des essences de Térébenthine," *Inst. du Pin Bul.*, No. **58**, 95(March, 1929)	34% α-pinene 62% β-pinene 1.6% dipentene 1.7% tails	0.8700^{15}	1.4712	−11.20°
Arbuzov, B., Abramov, A., and Valitova, F.	"A Study of Composition of Oleo Resin of *Pinus insignis*," *Zhur. Obshch. Khim.*, **2**, 376(1932)	40.6% α-pinene 56.4% β-pinene 0.3% camphene 3% high boiling fraction	$0.8685^{17.5}_{4}$	$1.4776^{14.5}$	− 3.25°
McCombs, T. H.	"Turpentine from Pines," *New Zealand J. Sci. Technol.*, **12**, 333(1931)	24% α-pinene 75% β-pinene 1% other subst.	0.872–0.874	.....	−12.4°

PINUS VIRGINIANA

Virginia pine (*Pinus virginiana*), also called scrub pine, grows in eastern United States from New Jersey and southeastern Virginia to Florida. It is a 2-needle pine, related to *P. radiata*, *P. attenuata*, *P. banksiana*, *P. muricata*, and other pines of the group Insignis. Recently this pine attracted the attention of pathologists who found that a certain fungus (of the genus *Fusarium*) causes an excessive flow of oleoresin (*4*). Experiments have been conducted in inoculating a healthy pine with the fungus in order to increase the flow of gum.

Oleoresin used in the present tests was received from Dr. George H. Hepting, Plant Pathologist of the Bureau of Plant Industry, stationed at the Southeastern Forest Experiment Station, Asheville, N. C. The oleoresin represented a mixed sample obtained from 9 trees treated with the fungus and 2 control (untreated). Upon distillation under reduced pressure, 23% of turpentine was obtained, with the following characteristics:

Density, d_4^{25}	0.8563
Index of refraction, $n_D^{25.5}$	1.4657
Specific rotation, $[\alpha]_D^{25}$	−3.83°

From the physical constants of the fractions (Table VII) it can be concluded that the oil consists mainly of *dl*-α-pinene (90%) with 8% *l*-α-pinene.

The nitrosochloride prepared from fraction 3 melted at 106–107°, and showed no depression of melting point when mixed with α-pinene nitrosochloride obtained from other sources.

SUMMARY

Turpentine was obtained under reduced pressure from oleoresin samples of the following species: *Pinus strobus*, *P. cembra*, *P. taeda*, *P. radiata*, and *P. virginiana*.

The oleoresin of *Pinus strobus* consisted of 75 per cent rosin and 25 per cent volatile oil (turpentine). The major constituents of the volatile oil are from *dl*-α-pinene (75 per cent) and *l*-β-pinene (15 per cent). Small amounts of terpene alcohols and ketones (4 per cent) and tricyclic sesquiterpene (0.3 per cent) were found.

The oleoresin of *Pinus cembra* consisted of 78.6 per cent rosin and 21.4 per cent turpentine. The turpentine contained 85 per cent *d*-α-pinene and 9 per cent *l*-β-pinene.

Pinus taeda oleoresin consisted of 85 per cent *d*-α-pinene and 12 per cent *l*-β-pinene. Limonene was not found.

The oleoresin of *Pinus radiata* consisted of 83 per cent rosin and 17 per cent turpentine. The

[1] Tapping of trees was done by Robert Roseman, at that time an employee at the Institute.

TABLE VI.—FRACTIONAL DISTILLATION OF THE VOLATILE OIL FROM *Pinus radiata*

Fraction	Distillation Range: Pressure, Mm.	Distillation Range: Temperature, °C.	Distillate, %	Density, d_4^{25}	Index of Refraction, n_D^{25}	Specific Rotation, $[\alpha]_D^{25}$
1	740	100–154	1	....	....	.....
2	740	154–155	27	0.8578	1.4690	− 1.73°
3	740	155–157	22	0.8587	1.4693	− 3.84°
4	740	157–158	11	0.8601	1.4718	− 7.57°
5	740	158–159	7	0.8628	1.4743	−11.90°
6	740	159–160	6	0.8659	1.4734	−13.40°
7	740	160–161	2	0.8666	1.4746	−14.47°
8	740	161–162	5	0.8638	1.4746	.....
9	740	162–163	15	0.8660	1.4748	−13°
10	740	163–169	2	....	....	.....
Residue	...	.....	2	....	....	.....

TABLE VII.—FRACTIONAL DISTILLATION OF THE VOLATILE OIL FROM *Pinus Virginiana*

Fraction	Distillation Range: Pressure, Mm.	Distillation Range: Temperature, °C.	Distillate, %	Density, d_4^{25}	Index of Refraction, n_D^{25}	Specific Rotation, $[\alpha]_D^{25}$
1	740	153–154	41	0.8552	1.4680	−2.36°
2	740	154–155	17	0.8543	1.4703	−5.50°
3	740	155–156	40	0.8546	1.4704	−4.66°
4	Residue	.....	2	....	....	.....

turpentine contained approximately 75 per cent *dl*-α-pinene and 22 per cent *l*-β-pinene.

Pinus virginiana oleoresin consisted of 77 per cent of rosin and 23 per cent of turpentine. The turpentine consists almost entirely of α-pinene (90 per cent of *dl*-α-pinene and 8 per cent of *l*-α-pinene).

Thus composition of turpentines of the five pine species studied is very simple, consisting in four cases chiefly, if not completely, of α- and β-pinenes and in one case of α-pinene alone.

Chemical composition of turpentine of *Pinus cembra* (*sibirica*) from Russia, as reported by Shkateloff, and that of *P. cembra* (a geographically separate tree) from Austria, as found by us, is apparently very similar. On the other hand, composition of *P. strobus* turpentine was found to be different from the morphologically closely related but geographically separated *P. monticola*. While these two species are difficult to distinguish by external characters, their turpentines are different inasmuch as *P. monticola* (from California) contains *n*-undecane and *P. strobus* does not. Analysis of *P. monticola* turpentine from Idaho is in progress.

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Composition of Gum Turpentine of *Pinus lambertiana**

By N. T. MIROV, A. J. HAAGEN-SMIT, and JAMES THURLOW

The composition of gum turpentine obtained from *Pinus lambertiana* has been determined and the results of the study are reported.

SUGAR PINE, or *Pinus lambertiana*, is the most majestic of all pines, reaching a height of 225 feet and a diameter of 10 feet. It grows from southern Oregon to lower California. In California, where it is best developed, it is found in mixture with other conifers at middle elevations of mountain ridges. Botanically it belongs to the subgenus *Haploxylon* or the white pines.

STUDIES

In 1913, turpentine of sugar pine growing at an elevation of 5800 feet was obtained by steam distilla-

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tion and analyzed by Schorger (1) with the following results:

Turpentine in oleoresin	16.4%
Density, d_{15}^{15}, of turpentine	0.8663
Index of refraction, n_D^{15}	1.4728
Specific rotation, $[\alpha]_D^{15}$	+10.42°

The composition of turpentine was reported as follows:

d-α-pinene	70–75%
β-pinene	about 5%
Probably phellandrene	2–3%
Probably aliphatic hydrocarbon	2–3%
A sesquiterpene of an aromodendrene type	

Oleoresin for the present work was obtained by the members of the Institute of Forest Genetics in 1947 in the Sierra Nevada Mountains, at an elevation of about 3500 feet. Twenty trees yielded, in two months, about 10,000 Gm. of honey-like oleoresin that remained liquid after prolonged storage. In some samples rosin acids precipitated reluctantly; in others they did not precipitate at all. Turpentine was obtained by distilling the oleoresin under reduced pressure. Toward the end of the distillation, the temperature inside the flask was 200° and the pressure was 0.02 mm.

The turpentine was distilled at atmospheric pressure until the temperature reached 187° and the distillation was then continued at 38 mm. until the temperature reached 183°. Finally the pressure was reduced to 0.1 mm. and the distillation was discontinued when the temperature reached 250°. The distillation characteristics of the turpentine and the physical constants of the fractions are listed in Table I.

Several high-boiling fractions were analyzed for carbon and hydrogen. The results of this analysis are listed in Table II.

The fractionation data show an accumulation of material at 154–162° and at 166–175° at atmospheric pressure. The presence of α-pinene in the interval 154–156° was shown by the preparation of *dl*-α-pinene nitrosochloride, having a melting point of 112°. No melting point depression was observed when the nitrosochloride was mixed with an authentic pinene nitrosochloride. The oxidation of fraction 164–166° gave nopinic acid, melting point 125°. From the fractionation data and the physical constants of the fraction we may conclude that the distillate contains approximately 65% *l*-α-pinene and 13% *l*-β-pinene.

Judging from its physical constants, fraction 11, boiling point 175–180°, contained a monocyclic

TABLE I.—FRACTIONAL DISTILLATION OF THE VOLATILE OIL FROM *Pinus lambertiana*

Fraction No.	Distillation Range: Pressure, Mm.	Distillation Range: Temperature, °C.	Distillate, %: Observed	Distillate, %: Cumulative	Density, d_4^{25}	Index of Refraction, n_D^{25}	Specific Rotation, $[\alpha]_D^{25}$
1	742	148–152	0.4	...	....	1.4581	.....
2	742	152–154	4.6	5.0	0.8536	1.4656	−15.52°
3	742	154–156	29.6	34.6	0.8536	1.4667	−16.56°
4	742	156–158	14.0	48.6	0.8541	1.4691	−17.75°
5	742	158–160	9.4	58.0	0.8554	1.4713	−19.12°
6	742	160–162	5.6	63.6	0.8554	1.4734	−19.24°
7	742	162–164	3.7	67.3	0.8565	1.4743	−19.62°
8	742	164–166	1.4	68.7	0.8577	1.4749	−18.72°
9	742	166–170	3.7	72.4	0.8577	1.4749	−17.30°
10	742	170–175	3.6	76.0	0.8577	1.4750	−16.37°
11	742	175–180	1.6	77.6	0.8536	1.4738	−12.12°
12	38	88–103	0.4	78.0	0.8774	1.4808	+ 0.43°
13	38	103–123	0.6	78.6	0.8995	1.4884	+12.94°
14	38	123–133	2.7	81.3	0.9169	1.4970	+27.84°
15	38	133–141	7.4	88.7	0.9187	1.4983	+32.49°
16	38	141–143	3.0	91.7	0.9187	1.4993	+33.30°
17	38	143–148	3.3	95.0	0.9169	1.4997	+31.36°
18	38	148–158	1.2	96.2	0.9164	1.5005	+25.30°
19	38	158–163	0.6	96.8	....	....	.....
20	38	163–183	0.7	97.5	....	....	.....
21	0.1	130–137	1.1	98.6	0.8937	1.4993	+ 7.35°
22	0.1	137–144	0.6	99.2	0.8995	1.5015	+ 4.38°
23	0.1	144–248	0.4	99.6	....	1.5165	.....
Residue	...	.....	0.4	100.0	....	....	.....

A measure of 5690 Gm. of oleoresin yielded 1045 Gm. of turpentine (or 18.4%), having the following characteristics:

Density, d_4^{24}	0.8669
Index of refraction, $n_D^{17.6}$	1.4753
Specific rotation, $[\alpha]_D^{17.6}$	−7.46°

Schorger's turpentine was dextrorotatory. In the present tests, 15 turpentine samples, obtained from individual trees, were analyzed. The specific rotation [α] varied from −18.25° to −1.35°. That is, all 15 samples were lævorotatory.

TABLE II.—CARBON-HYDROGEN ANALYSES OF DISTILLATION FRACTIONS OF *Pinus lambertiana* TURPENTINE

Fraction	Temperature Range, °C.	C, %	H, %	Total
14	123–133 at 38 mm.	86.16	11.59	97.75
15	133–141 at 38 mm.	87.24	11.96	99.20
16	141–143 at 38 mm.	87.63	11.70	99.03
17	143–148 at 38 mm.	87.84	11.81	99.65
20	163–183[a] at 38 mm.	83.39	11.54	94.93
21	130–137 at 0.1 mm.	87.70	11.95	99.65
22	137–144 at 0.1 mm.	87.66	11.97	99.63

[a] Semisolid.

terpene with two double bonds. This fraction did not yield any crystalline derivative with the methods used for the identification of dipentene, terpinene, and carene.

Fraction 12, boiling point 83–88° at 38 mm. or 181–187° at 740 mm., contains a mixture of hydrocarbon- and oxygen-containing derivatives. Terpineol, borneol, as well as other alcohols, were absent as shown by the Tschugaeff and Zerewitinoff (2) method. Saponifications showed the presence of only traces of ester. It is probable that this fraction contains some isomerization and oxidation products of pinene.

Fractions 15–18, boiling between 133 and 158° at 38 mm., contain hydrocarbon as seen from Table II. Attempts at preparing various halogen derivatives from these fractions were unsuccessful. Dehydrogenations were carried out on these fractions according to the method of Melville (3). The absence of any blue color in the dehydrogenated material showed the absence of azulenic sesquiterpenes. A phosphoric acid-petroleum ether partition confirmed this. Picrates were prepared from both the phosphoric acid portion and the petroleum ether portion. The melting points were found to be 114–115°, as compared to Melville's value of 115–116° for cadalene picrate.

The molecular refraction found for fractions 14, 15, 16, 17, and 18 are, respectively, 65.19, 65.24, 65.34, 65.50, and 65.61. For a sesquiterpene with 2 double bonds and 2 rings the molecular refraction, M_D, would be 66.1. These fractions contain, therefore, bicyclic sesquiterpenes of cadalene type structure.

Fraction 20, b. p. 163–183° at 38 mm., contained white crystals which could be separated from the oil on a porous plate. After recrystallization from ethyl alcohol, the crystals melted at 133°. Analysis showed that the crystals were composed of 80.67% of carbon and 11.94% of hydrogen. Calculated for a sesquiterpene alcohol $C_{15}H_{26}O$, the composition would be: carbon 81.08%; hydrogen 11.71%. Molecular weight of the crystalline substance was: found 213; calculated molecular weight for $C_{15}H_{26}O$, 229. Since this alcohol differs from all known sesquiterpene alcohols, the name Lambertol is introduced for this compound.

The fractions distilling at 0.1 mm. from 130–144° consist of hydrocarbons, probably diterpenes.

Saturated hydrocarbons were not present as shown by a treatment of the oil with fuming sulfuric acid.

SUMMARY

The volatile oil of *Pinus lambertiana* contains 65 per cent *l*-α-pinene, 13 per cent *l*-β-pinene, 10 per cent bicyclic sesquiterpene of cadalene type, 2 per cent sesquiterpene alcohol, $C_{15}H_{26}O$, m. p. 133° (Lambertol), and 2 per cent unidentified polyterpenes. It is seen that the results of the present investigation are at variance with the results obtained by Schorger. Although presence of α- and β-pinenes was verified, phellandrene and an aliphatic hydrocarbon were not detected. On the other hand, the sesquiterpene fraction was more fully explored.

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The Decomposition of Sodium Phenobarbital in Solutions of Aliphatic Amines and Alkanolamines*

By MELVIN F. W. DUNKER

The decomposition of aqueous solutions of sodium phenobarbital proceeds at room temperature in the presence of aliphatic amines and alkanolamines to produce the previously reported phenylethylacetylurea. The mixtures had *p*H values ranging between 10.2 and 10.6, whereas the aqueous solution of sodium phenobarbital had a *p*H of 9.2. In some cases crystals of the ureide formed within fifteen minutes after mixing the liquids.

IN CONNECTION with the reaction product of theophylline and phenobarbital, first noted with an aqueous solution containing aminophylline and sodium phenobarbital (1), it was called to the author's attention (2) that an aqueous solution of sodium phenobarbital containing ethylene diamine within a few days deposited white crystals melting at 147–148°. The instability of aqueous solutions of soluble phenobarbital and other barbiturates has been reported in several papers summarized by Husa and Jatul (3) On the basis of the melting point, mixed melting point with the ureide prepared directly from ethylphenylacetic acid, and analytical data, it was evident that the white crystalline solid was ethylphenylacetylurea previously reported as one of the decomposition products of sodium phenobarbital.

* Received Dec. 15, 1948, from the College of Pharmacy, Wayne University, Detroit, Mich.

Stabilized solutions of soluble phenobarbital and other barbiturates are reported in the literature (4–15), in many cases nitrogenous compounds of some type being present, such as amides (6–9), urethane (10, 11), alkanolamines (11), a water-soluble pyrazolone (12), and even diethylamine (13).

The stability of approximately 1.3 molar solutions of sodium phenobarbital in water at room temperature with the 22 amines listed in Table I was investigated. The *p*H values given for the resulting solutions may be considered as approximations since the Coleman *p*H meter was set against a buffer (*p*H 10) but no other correction was applied. The flasks containing *n*-propylamine, di-*n*-butylamine, *n*-amylamine, and 2-aminopentane developed crystals within ten to thirty minutes. The remainder of the solutions developed crystals in two to seven days, the solutions containing the alkanolamines requiring the longer periods. Lack of miscibility did not prevent the formation of crystals as may be noted by observing the behavior of the mixtures containing di-*n*-butylamine, 2-amino-octane, 2-ethyl-*n*-hexylamine, and di-*n*-butylaminoethanol.

EXPERIMENTAL[1]

To a solution of 5.08 Gm. (0.02 mole) sodium phenobarbital in 10 ml. water in a 25- or 50-ml. Erlenmeyer flask was added a mixture of 2 ml. water and 0.02 mole of amine (measured by pipette graduated in hundredths of a ml.). After thorough mixing, the *p*H of each homogeneous solution was immediately measured, using a glass electrode in a Coleman *p*H meter, the instrument being adjusted against a buffer having a *p*H of 10. In three cases (*n*-propyl- and *n*-amylamine and 2-aminopentane) crystals were beginning to form as the solution was being returned to the Erlenmeyer flask from the cup of the *p*H meter. The flasks were stoppered and set aside at room temperature (varying from 70° to 80° F.) for periods up to five weeks. The solution of sodium phenobarbital alone was observed over a period of four months. As crystals accumulated, the first crops were filtered off on Gooch funnels in two to ten days depending upon the rate of formation, the filtrate being returned to the flask. After thorough draining, the solid was washed with a small amount of cold water, the washing being added to the flask. The solid was partially dried by sucking air through it and then put in a desiccator over calcium chloride. After several days in the desiccator, the solids were weighed and the melting points taken. It was difficult to remove all of the amine from the solid in those cases where the mixture was not homogeneous. The residue of water-insoluble amine is no doubt responsible for the larger weights of solid obtained in these cases as well as the low melting points of the crystals obtained, usually in the first crop.

Up to four crops of crystals were filtered off over a period of up to five weeks in exactly the same way, depending on the rate of deposition. Crops of crystals having similar melting points were combined and recrystallized from hot ethanol. The material obtained from the mixtures in flasks 2 to 5, 7, 17 to 21, and 23 just as obtained from the reaction mixture, melted at or very near the melting point of ethylphenylacetylurea. All of these upon one recrystallization from ethyl alcohol melted at 148° ± 0.5° and gave no depression of melting point when mixed

[1] Melting points are uncorrected. The ethylenediamine was kindly supplied by Dr. L. A. Sweet of Parke, Davis & Co., and the remainder of the amines, except the triethanolamine, were supplied through the courtesy of Dr. P. W. Smith of Sharples Solvents.

TABLE I.—THE FORMATION OF PRECIPITATES IN SOLUTIONS OF SODIUM PHENOBARBITAL AND ALIPHATIC AMINES

Flask No.	Amine	Appearance	*p*H	First Appearance of Crystals	Total Wt. of Solid	Wt. of Solid M. 144–149°
1	.....	Homogeneous	9.2	2 days[a]	0.302	0.275
2	$(H_2N)_2C_2H_4$	Homogeneous	10.4	4 days	1.907	1.907
3	$C_2H_5NH_2$	Homogeneous	10.6	4 days	2.063	2.063
4	$(C_2H_5)_2NH$	Homogeneous	10.7	4 days	1.998	1.998
5	$(C_2H_5)_3N$	Homogeneous	10.6	5 days	1.866	1.866
6	n-$C_3H_7NH_2$	Homogeneous	10.6	10 min.	2.353	1.424
7	i-$C_3H_7NH_2$	Homogeneous	10.6	4 days	2.154	2.154
8	n-$C_4H_9NH_2$	Homogeneous	10.6	4 days	2.840	2.669
9	$(n$-$C_4H_9)_2NH$	2 layers	...	30 min.	2.508	0.431
10	s-$C_4H_9NH_2$	Homogeneous	10.6	4 days	2.912	1.989
11	i-$C_4H_9NH_2$	Homogeneous	10.7	5 days	2.224	1.996
12	n-$C_5H_{11}NH_2$	Homogeneous	10.8	30 min.	3.681	1.684
13	$C_2H_7(CH_3)CHNH_2$	Homogeneous	10.7	2 days	3.105	1.142
14	$(C_2H_5)_2CHCH_2NH_2$	Homogeneous	10.6	5 days	3.641	0.542
15	n-$C_6H_{13}(CH_3)CHNH_2$	2 layers	...	2 days	3.550	[b]
16	n-$C_4H_9(C_2H_5)CHCH_2NH_2$	2 layers	...	2 days	4.090	[b]
17	$(C_2H_5)_2NC_2H_4OH$	Homogeneous	10.4	4 days	1.282	1.282
18	$C_2H_5NHC_2H_4OH$	Homogeneous	10.4	3 days	1.710	1.710
19	$C_2H_5N(C_2H_4OH)_2$	Homogeneous	10.2	7 days	0.666	0.666
20	$CH_3CHOHC_2H_4NH_2$	Homogeneous	10.5	3 days	1.945	1.945
21	$(C_2H_5)_2NC_2H_4NH_2$	Homogeneous	10.6	3 days	2.230	2.230
22	$(n$-$C_4H_9)_2NC_2H_4OH$	2 layers	...	7 days	0.651	0.428
23	$(HOC_2H_4)_3N$	Homogeneous	10.0	7 days	0.453	0.453

[a] Only 3 crystals. After 1 month 0.200 Gm. of solid was filtered off.
[b] In both of these cases, several crystallizations were necessary to get sharp melting crystals. Some phenobarbital was isolated and considerable amounts of phenylethylacetylurea were obtained.

with a sample of ethylphenylacetylurea prepared from ethylphenylacetic acid by way of the acid chloride and recrystallized from ethyl alcohol.

A Kjeldahl determination on a sample of the ureide obtained in the ethylenediamine experiment gave the following values:

Theoretical for: $C_{11}H_{14}O_2N_2$, % N 13.59. Found: % N 13.06, 13.50.

The first crop of crystals from flask 6 weighed 0.92 Gm. and melted at 171–174°. Upon recrystallization from ethanol, the material melted at 173–174° and when mixed with U. S. P. phenobarbital, melted at 172–173°. The recrystallization of the solids obtained in flasks 15 and 16 also yielded some phenobarbital. An explanation of this finding is not immediately apparent.

SUMMARY

The decomposition of aqueous solutions of sodium phenobarbital in the presence of 22 aliphatic amines and alkanolamines at room temperature results in the formation of phenylethylacetylurea. The decomposition proceeds more slowly in the solutions of alkanolamines than in the solutions of the aliphatic amines.

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Evaluation of the Antibacterial Efficiency of Dilute Solutions of Free Halogens*,†

By LOUIS GERSHENFELD and BERNARD WITLIN

DILUTE SOLUTIONS of the halogens, in particular, free bromine, free iodine, and chlorine, have been recommended from time to time as general antiseptic washes to be used for wounds and as irrigations.

Due to the physical properties of the free halogens, they cannot be used in solutions of precisely similar constitution, yet the manners in which they are used are essentially comparable. Chlorine, because of high volatility, is customarily used as a hypochlorite, and in solution the Cl^- ion and the OCl^- ion are in such equilibrium as to permit the reaction: $HCl + HOCl \rightleftarrows Cl_2 + H_2O$ to occur. Br_2 and I_2 may be used in solution directly.

The composition of the solutions used in this study are similar to those reported in the literature and are those most likely to be employed in practice. They are a hypochlorite solution; a free bromine solution, a bromine-bromide solution, and an iodine-iodide solution. These formulas are the closest approach to a scheme where the three halogens can be compared on the same basis.

The first controlled experiments in wound healing were conducted by Carrel and Hartmann (1), Carrel (2), and DuNouy (3), who reported that wound healing occurred in a geometric curve. Recently Smelo (4), Anderson (5), and Arey (6) have confirmed the fact that wound healing followed a regular predictable course, and that infection is the most important factor in delaying wound healing.

Prior to the introduction of antiseptic surgery, preparations of free bromine were employed for gangrenous infections (7); and dilute solutions were used for certain types of respiratory infections (7).

Carrel pioneered the irrigation technique for deep infected wounds with Dakin (dilute sodium hypochlorite) solution and later with Dichloramine-T (8–10). Daufresne (11) and others (12, 13) also employed hypochlorous acid.

LaWall and Tice (14) recommended the use of a dilute solution of iodine in isotonic solution of sodium chloride for irrigation purposes.

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Babcock (15, 16) reported on the use of dilute bromine solutions for use as wet compresses and for the irrigation of odorous wounds and fistulas.

The experimental studies on the use of dilute solutions of free halogens, as reported in the literature, have been conducted on each halogen independently; furthermore, different techniques were employed in the investigations. The purpose of this study was to compare the *in vitro* bactericidal efficiencies of dilute solutions of free bromine, free iodine, and chlorine under identical test conditions.

EXPERIMENTAL

Stock solutions of the free halogens were prepared as described in the following paragraphs.

Chlorine.—Solutions of chlorine (as sodium hypochlorite) 1:5000 (0.02%) were prepared by diluting a sodium hypochlorite solution containing 5% available chlorine to the desired concentration with distilled water. The solutions were standardized as to available chlorine content in accordance with the A. O. A. C. procedure (17).

Bromine.—Solutions of free bromine, 1:5000 (0.02%) by weight, were prepared by placing 0.2 Gm. of liquid bromine in a glass-stoppered liter volumetric flask, adding distilled water to 1000 cc., and mixing until solution was effected.

Iodine.—Solutions of free iodine 1:5000 (0.02%) were prepared by diluting 2% Iodine Solution (N. F. VIII) and/or 2% Iodine Tincture (U. S. P. XIII) to 0.02% (1:5000) concentrations with distilled water.

Another series of 0.02% solutions of free bromine, free iodine, and chlorine were prepared as above using isotonic solution of sodium chloride (0.9%) instead of distilled water as the diluent.

(A) Bactericidal Efficiencies of Various Solutions as Determined by a Modified F. D. A. Technique

The bactericidal efficiencies of the solutions of free bromine, free iodine, and chlorine were determined at 24° and 37° by a modified F. D. A. technique (18) employing twenty-four-hour-old cultures of *Staphylococcus aureus*, *Salmonella* (*Eberthella*) *typhosa*, *Pseudomonas aeruginosa*, *Escherichia coli*, vegetative forms of *Bacillus mesentericus*, vegetative forms of *Bacillus megatherium*, and vegetative forms of *Bacillus subtilis* as the test organisms. The only modification of the F. D. A. technique was the additional use of shorter time intervals for the first five minutes of the test. Standard F. D. A. loopfuls (18) were transplanted into 10-cc. quantities of sterile F. D. A. broth at one-, two-, five-, ten-, and fifteen-minute intervals. Transplants were incubated for forty-eight hours at 37° and the presence or absence of growth was recorded.

Tests for free halogens were performed at the conclusion of the time-testing period. Free chlorine was detected by the addition of acidified starch-potassium iodide solution and also with orthotolidine. Free bromine was detected by the addition of an excess of potassium iodide to the solution and the liberated iodine detected with starch test solution (U. S. P.) (19). The presence of free iodine was noted by the use of starch test solution (U. S. P.).

Findings.—Five cubic centimeters of 1:5000 (0.02%) chlorine were ineffective within fifteen minutes against 0.5 cc. of twenty-four-hour cultures of the above-mentioned bacteria at 24° and 37°.

Five cubic centimeters of free bromine in the concentrations listed below were effective at 24° within one minute against 0.5 cc. of twenty-four-hour cultures of the following organisms:

S. aureus, 1:6000; *S. (E.) typhosa*, 1:10,000; and *E. coli*, 1:7500; but ineffective within fifteen minutes against the other organisms mentioned above. Five cubic centimeters of 1:5000 (0.02%) free bromine were effective only against *S. aureus* within one minute at 37°, but ineffective within fifteen minutes at 37° against the other above-mentioned organisms.

Five cubic centimeters of the following concentrations of 1:5000 (0.02%) free iodine were effective within one minute at 24° and 37° against 0.5 cc. of twenty-four-hour cultures of the following test organisms:

	24°	37°
S. aureus	1:12,500	1:20,000
S. (E.) typhosa	1: 9,000	1:12,500
E. coli	1:12,500	1:18,000
P. aeruginosa	1:10,000	1:15,000
B. mesentericus (vegetative)	1: 7,500	1:10,000
B. megatherium (vegetative)	1: 7,500	1:10,000

Five cubic centimeters of 1:5000 (0.02%) iodine were ineffective against 0.5 cc. of a twenty-four-hour culture of *B. subtilis* (vegetative) within fifteen minutes at both 24° and 37°.

(B) Bactericidal Efficiencies of Dilute Solutions of Free Bromine, Free Iodine, and Chlorine in the Presence of Citrated Human Plasma

Solutions of free bromine, free iodine, and chlorine were prepared in 1:5000 (0.02%) concentrations in isotonic solution of sodium chloride containing 5% of human citrated plasma. Another series of 1:5000 (0.02%) solutions of the free halogens were prepared as above with isotonic solution of sodium chloride containing 10% of human citrated plasma.

Bactericidal efficiency tests were conducted at both 24° and 37° using the same test bacteria as given under (A).

Findings.—Five cubic centimeters of solutions of 1:5000 (0.02%) chlorine in the presence of 5% and 10% citrated human plasma were ineffective against 0.5 cc. of twenty-four-hour cultures of *S. aureus*, *S. (E.) typhosa*, *P. aeruginosa*, *E. coli*, *B. mesentericus* (vegetative), *B. megatherium* (vegetative), and *B subtilis* (vegetative) within fifteen minutes at 24° and at 37°.

Five cubic centimeters of solutions of 1:5000 (0.02%) bromine (w/v) were capable of killing 0.5 cc. of twenty-four-hour cultures of *S. aureus*, *S. (E.) typhosa*, and *E. coli* in the presence of 5% citrated human plasma within one minute at 24°.

Bromine solutions (1:5000) were ineffective against twenty-four-hour cultures of all of the bacteria tested (under A) at 24° in the presence of 10% citrated human plasma and at 37°[1] in the presence of either 5% or 10% citrated human plasma.

Free Iodine.—Five cubic centimeters of solutions of free iodine 1:5000 (0.02%) were effective at 37°

[1] Bromine appears to exert greater bactericidal efficiency at room temperature (24°) than at body temperature (37°). This is probably due to the instability of bromine at higher temperatures.

against 0.5 cc. of *S. aureus*, *S.* (*E.*) *typhosa*, *E. coli* and *P. aeruginosa* in the presence of 5% citrated human plasma. Half cubic-centimeter quantities of *S.* (*E.*) *typhosa* and *E. coli* were the only organisms under test killed within one minute at 24° upon exposure to 5 cc. of 1:5000 (0.02%) free iodine in the presence of 5% citrated human plasma. *S.* (*E.*) *typhosa* and *E. coli* were not killed within fifteen minutes at 24° by 1:5000 (0.02%) iodine in the presence of 10% citrated human plasma. *B. mesentericus* (vegetative), *B. megatherium* (vegetative), and *B. subtilis* (vegetative) were not killed by free iodine 1:5000 (0.02%) in the presence of 5% or 10% citrated human plasma at 37° or 24° within fifteen minutes.

(C) Effect of *p*H

Solutions of free bromine, free iodine, and chlorine were freshly prepared in 1:5000 (0.02%) concentrations in McIlvaine's and in Sørensen's buffer solutions at *p*H 2.2, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0.

Five cubic centimeters of the solutions were tested for bactericidal efficiency at 37° against 0.5 cc. of twenty-four-hour cultures of the test organisms (see under A). The buffer solutions at varying *p*H values were likewise tested for bactericidal efficiencies.

Findings.—Fresh solutions of chlorine (1:5000) and fresh solutions of free bromine (1:5000 *w/v*) prepared in McIlvaine's or Sørensen's buffer solutions at *p*H 2.2, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 were not effective against *S. aureus*, *S.* (*E.*) *typhosa*, *P. aeruginosa*, *E. coli*, *B. mesentericus* (vegetative), *B. megatherium* (vegetative), and *B. subtilis* (vegetative) at 37°.

Freshly prepared free iodine solutions (1:5000) at *p*H 2.2, 3.0, 4.0, and 5.0 killed *S. aureus*, *S.* (*E.*) *typhosa*, *E. coli*, *P. aeruginosa* and *B. megatherium* (vegetative) within one minute. *S. aureus* and *S.* (*E.*) *typhosa* were killed within one minute at *p*H 6.0, 7.0, and 8.0 and *E. coli* at *p*H 6.0 and 7.0. *B. mesentericus* (vegetative) was killed within one minute at *p*H 2.2, 3.0, and 4.0. *B. subtilis* was not killed by 0.02% free iodine (1:5000) within the *p*H range of 2.2 to 8.0.

None of the test bacterial cultures used were killed within fifteen minutes by the buffer solutions at *p*H 2.2, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 under conditions of the test.

CONCLUSIONS

I. Bactericidal efficiency tests of dilute solutions (1:5000) of the free halogens prepared either in distilled water or in an isotonic solution of sodium chloride revealed the data presented below.

Chlorine.—Five cubic centimeters of a 1:5000 (0.02%) solution of chlorine were ineffective at both 24° and at 37° against 0.5 cc. of twenty-four-hour cultures of *S. aureus*, *S.* (*E.*) *typhosa*, *P. aeruginosa*, *E. coli*, *B. mesentericus* (vegetative), *B. megatherium* (vegetative), and *B. subtilis* (vegetative).

Free Bromine.—Five cubic centimeters of a 1:5000 solution of free bromine were capable of killing 0.5 cc. of twenty-four-hour cultures of *S. aureus*, *S. typhosa*, and *E. coli* at 24° but ineffective against the remainder of the test organisms (listed under "Chlorine") within fifteen minutes at 24°.

At 37°, 5 cc. of a 1:5000 (0.02%) free bromine solution were capable of killing 0.5 cc. of a twenty-four-hour culture of *S. aureus* within one minute but were ineffective against the other test organisms (listed under "Chlorine") within fifteen minutes.

Free Iodine.—Five cubic centimeters of free iodine solutions 1:5000 (0.02%), prepared by diluting U. S. P. Iodine Tincture or Iodine Solution (N. F.) with distilled water or isotonic solution of sodium chloride, were effective within one minute at both 24° and at 37° against 0.5 cc. of twenty-four-hour cultures of *S. aureus*, *S.* (*E.*) *typhosa*, *E. coli*, *P. aeruginosa*, *B. mesentericus* (vegetative), and *B. megatherium* (vegetative), but were ineffective against *B. subtilis* (vegetative) under the same conditions.

II. Solutions of free bromine, free iodine, and chlorine were prepared in 1:5000 (0.02%) concentrations with distilled water or in isotonic solution of sodium chloride each containing either 5% or 10% of citrated human plasma. The following findings were disclosed.

Chlorine.—Chlorine was ineffective against *S. aureus*, *S.* (*E.*) *typhosa*, *P. aeruginosa*, *E. coli*, *B. mesentericus*, *B. megatherium*, and *B. subtilis* in the presence of 5% and 10% citrated human plasma.

Free Bromine.—Five cubic centimeters of 1:5000 (0.02%) bromine solutions (*w/v*) were capable of killing 0.5 cc. of twenty-four-hour cultures of *S. aureus*, *S.* (*E.*) *typhosa*, and *E. coli* within one minute at 24° in the presence of 5% citrated human plasma, but were ineffective against the same organisms at 37°. Five cubic centimeters of 1:5000 bromine in 5% citrated human plasma were ineffective against 0.5 cc. of *P. aeruginosa*, *B. mesentericus* (vegetative) *B. megatherium* (vegetative), and *B. subtilis* (vegetative) at 24° and at 37°.

Five cubic centimeters of 1:5000 bromine were ineffective at 24° and 37° against all the test organisms in the presence of 10% citrated human plasma.

Free Iodine.—Five cubic centimeters of iodine 1:5000 within one minute killed 0.5 cc. of cultures of *S. aureus*, *S.* (*E.*) *typhosa*, *P. aeruginosa*, and *E. coli* in the presence of 5% citrated human plasma at 37°. Half cubic-centimeter quantities of *S.* (*E.*) *typhosa* and *E. coli* were killed within one minute at 24° when exposed to 5 cc. of 1:5000 iodine in the presence of 5% citrated human plasma, but not in the presence of 10% citrated human plasma. *B. mesentericus* (vegetative), *B. megatherium* (vegetative), and *B. subtilis* (vegetative) were not killed by iodine 1:5000 in the presence of 5% or 10% citrated human plasma at 24° or 37° within fifteen minutes.

III. Solutions of free bromine, free iodine, and chlorine, prepared in 1:5000 concentrations in McIlvaine's and Sørensen's buffer solutions (20) at varying *p*H values (2.2, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0) revealed the following: Five cubic-centimeter solutions of chlorine 1:5000 and solutions of free bromine 1:5000 prepared in buffer solutions at *p*H values of 2.2, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 were ineffective at 37° against 0.5 cc. of twenty-four-hour cultures of *S. aureus*, *S.* (*E.*) *typhosa*, *P. aeruginosa*, *E. coli*, *B. mesentericus* (vegetative), *B. megatherium* (vegetative), and *B. subtilis* (vegetative).

Five cubic centimeters of 1:5000 free iodine solutions buffered at *p*H 2.2, 3.0, 4.0, and 5.0 killed *S. aureus*, *S.* (*E.*) *typhosa*, *E. coli*, *P. aeruginosa*, and *B. megatherium* (vegetative) in one minute at 37°. In addition *S. aureus* and *S.* (*E.*) *typhosa* were killed within one minute at *p*H 6.0, 7.0, and 8.0 and *E.*

coli at *p*H 6.0 and 7.0. *B. mesentericus* (vegetative) was killed in one minute at *p*H 2.2, 3.0, and 4.0. *B. subtilis* (vegetative) was not killed by 1:5000 free iodine within the *p*H range of 2.2 to 8.0.

SUMMARY

Iodine solutions in concentrations of 1:5000 (0.02 per cent) in either distilled water or isotonic solution of sodium chloride or either of the two diluents each containing 5 per cent citrated human plasma, displayed more effective antibacterial activity against several test bacteria either at 24° or 37° as compared to solutions of chlorine (1:5000) or free bromine (1:5000 *w/v*). The test bacteria used were broth cultures of: *Staphylococcus aureus, Salmonella (Eberthella) typhosa, Pseudomonas aeruginosa, Escherichia coli*, vegetative forms of *Bacillus megatherium*, vegetative forms of *Bacillus mesentericus*, and vegetative forms of *Bacillus subtilis*.

Weak iodine solutions (1:5000) displayed findings as above in *p*H ranges from 2.2 to 8.0. Identical concentrations of free bromine and chlorine under similar tests were ineffective.

Five-cubic centimeter quantities of McIlvaine's and Sørensen's buffer solutions (as controls) at *p*H 2.2, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 did not kill 0.5-cc. quantities of the test bacterial cultures within fifteen minutes at 24° and 37°.

Solutions of free iodine 1:5000 (0.02 per cent) for use as an antiseptic wash or for irrigations were readily prepared by diluting 1 cc. of 2 per cent Iodine Solution (N. F. VIII) and/or 2 per cent Iodine Tincture (U. S. P. XIII) to 100 cc. with distilled water or isotonic solution of sodium chloride.

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Book Reviews

An Introduction to Materia Medica and Pharmacology. By ELSIE E. KRUG and HUGH ALISTER MCGUIGAN, 5th ed. C. V. Mosby Co., St. Louis, 1948. 558 pp. 13.5 x 21.5 cm. Price $4.

Pharmacology and Therapeutics in Nursing. By MARION S. DOOLEY and JOSEPHINE RAPPAPORT. McGraw-Hill Book Co., New York, 1948. xi + 444 pp. 15 x 23.5 cm. Price $3.75.

To determine how much pharmacology to teach a nurse is not easy. Such a decision may depend upon whether she is considered to be a person in the professional or subprofessional category. Modern nursing schools with their added emphasis upon the teaching of academic subjects would seem to suggest that she is considered professional.

If the nurse is to have status as a professionally trained person, she should have a thorough groundwork in the principles of drug action and she must know the main and side actions of drugs to a far greater extent than either of these books suggests. With the increasing number of new drugs becoming available every month and with most hospitals participating in the clinical testing of new drugs, more responsibility is placed in the nurse's hands than ever before. She does not need the detailed knowledge of the medical student, but it would seem that she needs more information than either of these books gives.

The reviewer believes that the Dooley-Rappaport text is restricted to too few drugs. For example, only the official barbiturates are considered, despite the fact that the nurse will be confronted with many commercial barbiturates. It is important for her to be familiar with the names, doses, and the type or category to which these barbiturates belong.

The Krug-McGuigan text, on the other hand, is a little less discriminating in the selection of drugs, but is not quite so careful in the accuracy of its facts. For example, on Page 149, nicotinamide is said to be the same as coramine (which is diethylnicotinamide) and is said to have a nicotine-like action.

Its structural similarity to nicotine is emphasized despite the fact that the compound has been renamed niacinamide in order to de-emphasize this structural similarity.

There are a number of groups—nurses, veterinarians, pharmacists and dentists, to name a few—who are interested in pharmacology from a different viewpoint than that of the physician. It is to be hoped that some day the "ideal" book will be available for these groups.

Surface-Active Agents—Their Chemistry and Technology. By ANTHONY M. SCHWARTZ and JAMES W. PERRY. Interscience Publishers, New York, 1949. xi + 579 pp. 15 x 23 cm. Price $10.

During the past decade there has been a greatly increased interest in surface-active agents and surface chemistry. Although there have been many books written in this field, most of them have been written from the "practical" viewpoint and have been incomplete in background information. This book by Schwartz and Perry combines the theoretical and the practical in a most effective manner.

In an introductory chapter the authors summarize general information concerning surface chemistry very briefly. This is followed by a section on processes for synthesizing and manufacturing surface-active agents. In this section the surface-active agents are divided into chemical types and each type is discussed from the viewpoint of the laboratory synthesis and commercial production. This section, as is the entire book, is well documented. Especially helpful are the many references to the patent literature.

The second division of the book concerns the physical chemistry of surface-active agents. Here one finds discussions on surface and interfacial relationships of pure liquids, surface properties of solutions, the bulk properties of surface-active solutions, the gross effects and technical evaluation of such agents, and the relationship of surface activity to chemical constitution discussed in an admirable manner. Although the facts and theories are concisely expressed, nothing of importance has been omitted.

The last portion of the book is given over to the practical aspects of surface chemistry. Of particular interest to pharmacists are chapters on "cosmetics and personal use" and "pharmaceutical, germicidal, fungicidal, and disinfectant uses."

A full author and subject index complete the book.

This is the book on surface-active agents that pharmaceutical chemists have been looking for. The only thing that it lacks is a list of the characteristics of trade-named products and their manufacturers.

Industrial Hygiene and Toxicology. Volume I. Edited by FRANK A. PATTY. Interscience Publishers, Inc., New York, 1948. xxvii + 531 pp. 16 x 25 cm. Price $10.

Industrial hygiene, having awakened widespread interest since the 1920's, is a relatively new field in the United States. In Europe, it dates back farther, having interested Paracelsus, among others. Despite its youth, it has made rapid strides, however, both in the assembling of large masses of data and in broadening its scope. It is fitting that this excellent reference, by many authorities, should become available at this time.

Much of the earlier work in the field of industrial hygiene revolved around toxic gases and fumes, industrial dusts, and machinery guards to prevent maiming. Modern industrial hygiene has gone far beyond this treatment phase into prophylaxis. The industrial hygienist now seeks to inculcate positive health into the worker so as to promote efficiency and well-being. For this reason it is not surprising to find the chapters by Josef Brozek and W. N. Witheridge on the effects of personal factors and environmental factors on competence and fatigue.

Frank A. Patty, the editor, has written the introductory chapter on "retrospect and prospect," as well as a chapter on the survey and personnel and chapters on the mode of entry and action of toxic materials, sampling and analysis of atmospheric contaminants, and respirators and respiratory protective devices.

Pharmacists will be particularly attracted to the chapter by Dr. Louis Schwarz on occupational dermatosis, for in this section will be found a discussion on the use of protective ointments. Pharmacists in industrial areas frequently can cooperate with the industrial hygienists in the development of such ointments and creams.

Other important chapters found in the first volume of this two-volume reference deal with the physiological effects of abnormal atmospheric pressure, radiant energy and radium, ventilation, the visible marks of occupation and occupational diseases, fire and explosion hazards of combustible gases and vapors and combustible dusts, and the role of dust in the causation of occupational disease.

To those with the responsibility of the health of relatively large numbers of people, this book will be of interest. It is helpful, not only for the factual information, but for the investigative approach consistently maintained.

We are looking forward to Volume II.

Newer Synthetic Analgesics. Consulting Editor, M. L. TAINTER. New York Annals of the New York Academy of Sciences. New York, 1948. 174 pp. 15 x 24 cm. Price $2.75.

The New York Academy of Sciences is famous for the high quality and practicality of its symposia. During 1948 one of the outstanding symposia was that on newer synthetic analgesics, the subject of this issue of the *Annals*.

This symposium was particularly timely because several outstanding drugs have appeared in this field recently and new developments in testing have also come upon the scene.

The symposium opened with an excellent historical review on pain by Dr. M. L. Tainter. The chemistry of natural and synthetic analgesics was then reviewed by Dr. Lyndon F. Small. Dr. Carl C. Pfeiffer and co-workers next reviewed experimental methods for studying analgesia. Dr. Lloyd C. Miller gave a critique of analgetic testing methods.

The more general papers on chemistry and pharmacology were followed by papers dealing with

specific drugs such as Methadone, Metopon, Dermerol, and related compounds. Such eminent pharmacologists as Nathan B. Eddy, F. F. Yonkman, and K. K. Chen presented the papers in this group.

The next group of papers dealt with the addiction problem and were presented by M. H. Seevers, Harris Isbell, and Robert C. Batterman.

Dr. E. E. Nelson and Mr. H. J. Anslinger, respectively, discussed the relations of the Food and Drug Administration and the Bureau of Narcotics to new analgesic agents.

The final papers dealt with clinical application of analgesics and were presented by Raymond N. Bieter, E. A. Rovenstine, Bert B. Hershenson, R. W. Houde, and others.

All papers presented and bound together in this issue of the *Annals* were of exceptionally high quality. An amazing amount of information is covered on these pages. The reader is brought up to date in this important field in an effective and interesting manner. Almost uniformly the authors have pointed out gaps in our knowledge, thus stimulating the research worker.

The Academy is to be congratulated upon its choice of subject matter and authors for this excellent symposium.

Pharmacologic Principles of Medical Practice. By JOHN C. KRANTZ, JR., and C. JELLEFF CARR. Williams & Wilkins Co., Baltimore, Md., 1949. xv + 980 pp. 15 x 23 cm. Price $10.

Probably no supporting science to the field of medicine has made greater progress during the past decade than pharmacology. Not only have a wealth of new drugs entered the picture, but the drugs have been of such a nature that whole concepts of drug action have been changed or, at least, have been modified. Because of these advances, a new textbook has been badly needed and Drs. Krantz and Carr have met that need very well.

The authors have consistently emphasized their theme of pharmacologic *principles* in medical *practice*. Never is basic principle sacrificed for obscure detail and yet rarely absent is the detail necessary for intelligent practice. In addition, the facts are marshaled in a readable and teachable manner. The human interest approach is used throughout and given emphasis by an excellent collection of portraits of the leaders in the field—Magendie, Abel, Fleming, Spalding (too few pharmacologists appreciate his contribution), A. J. Clark, Waksman, Domagk, Wells, Sertürner, Chen, Hunt, McCollum, and others.

The drugs are grouped according to physiologic processes into antiinfective drugs, the response of skin and mucous membranes to drugs, the response of the central nervous system, the autonomic nervous system, the heart and circulation, and the reproductive system to drugs, and the effect of pharmacologic agents on metabolism.

The appendix carries an interesting section on the discovery and evaluation of new drugs as well as a series of typical prescriptions.

The introduction contains the usual introductory information—scope, history, nature and source of drugs, drug control, the prescription, general principles of drug action, and much more. Many phases of this section are difficult to handle in a brief and fair manner. The authors have been essentially factual although their interpretation of the position and scope of the National Formulary is not quite accurate. It is not true that drugs which are prescribed at least 10,000 times a year are admitted to the National Formulary automatically, although extent of use is an important factor in determining scope. The authors imply that the National Formulary is in a secondary position relative to drug standards, but the reviewer maintains that, while its scope is different from that of the United States Pharmacopœia, the book is no less important. From the viewpoint of public health, it would seem to be just as important that drugs which are widely used should be standardized even though they may not bear the stamp of approval of the Pharmacopœia.

Farmaseuttisia Ja Kemiallisia Synonymeja. By Y. W. JALANDER. Helsingfors, 1948. xxvii + 570 pp. + 47-p. supplement. 12 x 19 cm.

This book comprises a list of about 21,000 synonyms intended for the use of the Scandinavian countries, more particularly Finland. A table of comparative acid strengths of the various countries throughout the world is included also.

Although the reviewer believes this book to be of inestimable value to the pharmacists for whom it is written, it may be of limited interest only to American pharmacists who are interested in foreign drugs and foreign prescriptions.

Basic Facts of Health Education for Members of the Medical, Pharmaceutical and Nursing Professions. The Pharmaceutical Press, 17 Bloomsbury Square, London, W.C.I., England. vi + 193 pp. 13.5 x 21 cm. Price 7*s.* 6*d.*

Basic Facts of Health Education is a collection of essays selected from British Ministry of Health Bulletins and which appeared in the *Pharmaceutical Journal* (London) during 1944–1947. These Bulletins were supplied to British pharmacists to keep them informed of developments about which they might be consulted by the general public and to assist them in the dissemination of useful information.

From the scope of the topics covered it is evident that the British, too, are becoming increasingly aware of the significance of the pharmacist as a source of public health information. Some of the topics covered are communicable diseases like measles, chicken pox, and scarlet fever, all of which are discussed in separate essays in an authoritative and clear manner.

Some other interesting topics presented are antenatal care: its scope and extent; the work of a medical officer of health; the importance of pure water supplies; food poisoning; cancer—some facts about treatment and early symptoms; dysmenorrhea; notes on constipation remedies; eradication of fleas in wartime; mass miniature radiography; coughs and cough mixtures; stored blood and plasma; geriatrics; and many other cogent subjects.

Many American pharmacists will wish to have a copy of this handbook at their prescription counters since it contains much useful information on health education.

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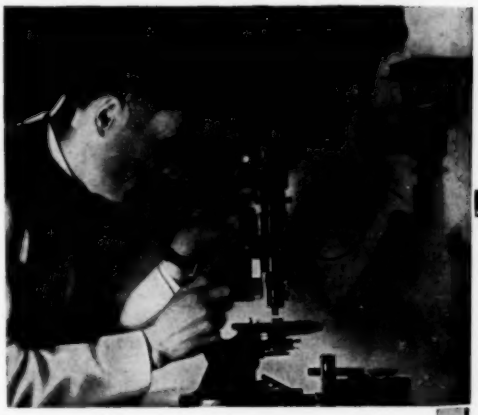

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